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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

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MEMORANDUM

Subject: Piperonyl Butoxide: Revised Metabolism Assessment Review Committee Report

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This document contains error correction revisions only. Any substantive corrections will be corrected after the public comment period.

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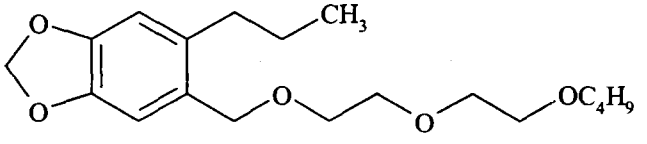
1. Introduction

Piperonyl butoxide is currently exempt from the requirement for a tolerance for residues when applied to the growing crop; however, the exemption does not apply when piperonyl butoxide is applied to a crop at the time of or after harvest [40 CFR §180.1001(b)(4)]. Tolerances for residues of piperonyl butoxide in/on various plant commodities, as a result of postharvest use, and in animal commodities are currently expressed in terms of piperonyl butoxide, [(butylcarbityl)(6-propyl piperonyl)ether] [40 CFR §180.127]. Adequate analytical methods are available for enforcement of tolerances for milk and animal tissues. These GC/FID/ECD methods (Methods I and Methods A and B) recover piperonyl butoxide *per se* and are published in PAM Vol. II Section 180.127.

Piperonyl butoxide is a FIFRA List B pesticide active ingredient classified as a synergist. Synergists are chemicals which, while lacking pesticide properties of their own, enhance the pesticidal properties of other active ingredients. As a synergist, piperonyl butoxide works by inhibiting the detoxification of the pesticide by the insect pests. Piperonyl butoxide was first developed around 1947 using naturally occurring safrole as the key raw ingredient. It is presently registered for use in combination with a wide variety of insecticides such as pyrethrins, allethrins, permethrin, tetramethrin, rotenone, and carbamates.

TABLE 1. Piperonyl Butoxide Nomenclature

Compound	Chemical Structure
	
Common name	Piperonyl butoxide
IUPAC name	5-[2-(2-butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole or 2-(2-butoxyethoxy)ethyl 6-propylpiperonyl ether
CAS name	5-[[2-(2-butoxyethoxy)ethoxy]methyl]-6-propyl-1,3-benzodioxole
CAS #	51-03-6
End-use products	Refer to the Residue Chemistry Chapter of the Piperonyl Butoxide RED (DP Barcode D288366) for a list of end-use products with food/feed uses.

Issue for the Committee

Does the committee agree that the residue of concern for the tolerance expression and for risk assessment should be piperonyl butoxide and that this is the only residue of concern for target crops, rotational crops, livestock, and drinking water?

2. MARC Decision

Table 2. HED MARC Meeting Summary Chart

Chemical: Piperonyl Butoxide		
Date: 28-April-2004		
Residues of Concern		
Matrix	For Risk Assessment	For Tolerance Expression
Plants	2X the Parent	Parent only
Livestock	Parent only	Parent only
Rotational crops	No decision due to lack of data	No decision due to lack of data
Water	Parent, PBO alcohol, PBO aldehyde, PBO acid	N/A

Rationales:

Plants:. Metabolism studies conducted on lettuce, cotton, and potato indicated that parent is the only major residue (>10% total radioactive residue (TRR)). However, all the plant metabolism studies are poorly conducted in that only 20 - 40 % of the TRR were successfully identified. Only the lettuce study firmly identified any metabolites. The latter represented conjugates in which the polyether side chain had been hydroxylated or cleaved at one of the ether linkages. These conjugates are similar to some metabolites observed in the rat. MARC does not believe that these metabolites will be significantly more toxic than the parent, but can not exclude them as being significantly less toxic. Since these metabolites were present in lettuce at about the same level as the parent, MARC suggested that the risk assessment team use 2X the parent residue levels for risk assessment, unless field trial data on metabolites on related crops indicate as lower ratio is appropriate. For tolerance expression, parent only is adequate to serve as the misuse indicator.

Livestock: The metabolism of PBO in lactating goat and hen poultry following dermal administration indicated that parent is the major metabolite (>30% TRR except for goat kidney and liver). There are no specific toxicity concerns for all metabolites, which with few exceptions were present at <10% TRR. Since the major route of exposure would most likely come from dermal treatment of livestock with piperonyl butoxide, MARC concluded that parent only is the residue of toxicological concern to be included in risk assessment and tolerance expression .

Drinking Water: Environmental fate studies indicated that PBO is moderately mobile in soil-water systems. Lab studies indicated that Piperonyl butoxide degrades in the environment by photolysis in water (half-life 8.4 hours), and is metabolized by soil microorganisms (half-life 14 days). The aqueous photolysis route may be significant for PBO due to its use in mosquito control applications over intermittently flooded areas. The major degradates are PBO-alcohol (54.7% the applied dose in photodegradation in water, and 44% of the applied dose in photodegradation in soil), PBO-aldehyde (11.6% of the applied dose in photodegradation in

water, and 7.6% of the applied dose in photodegradation in soil), and PBO-acid (9.8% of the applied dose in photodegradation in soil and 17% of the applied dose in aerobic soil). These degradates are expected to be more soluble in water and therefore more mobile in soil-water systems than the parent, based on their lower molecular weights and hydrophilic moieties. Based on the structural similarity, MARC believes that these three degradates will likely share the same toxicity as the parent, and therefore, MARC recommended to include these three degradates into drinking water assessment. There are no specific toxicity concerns for all other minor metabolites. One theoretical degradate over which MARC had concern was butyl carbitol, or diethylene glycol butyl ether, which would be formed when PBO cleaves to generate PBO-alcohol. Literature search in *Chemosphere* (1998, risk assessment for glycol ethers) indicated that it is not persistent in the environment based on OECD biodegradation tests, and therefore not of concern for drinking water.

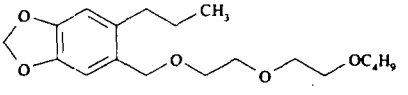
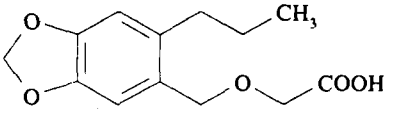
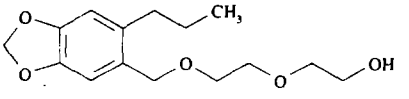
Members attended: Abdallah Khasawinah, Yan Donovan, Norman Birchfield, Rick Loranger, Christine Olinger, John Doherty, Alberto Protzel, Leonard Keifer.

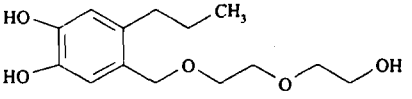
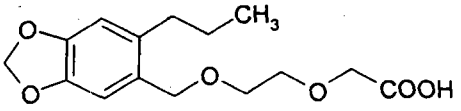
Members in Absentia: PV Shah, Pauline Wagner, Leung Cheng, Bill Wassell.

Alternate Members attended: None

Non Members: Thurston G. Morton, Santhini Ramasamy, William P. Eckel, Susan Hummel

Table 3. Piperonyl butoxide and its metabolites in lactating goats (MRID 43643201) and laying hens (MRID 43712601).

Common Name Chemical Name	Structure	Substrate [a]
Piperonyl butoxide (Butylcarbityl)(6-propyl piperonyl)ether		Goat milk, fat, kidney, liver, leg muscle,[b] and loin muscle [b] Poultry egg yolk, egg white, fat, liver,[b] thigh muscle,[c] and skin
Metabolite 11 1-(6-Propyl-1,3-benzodioxol-5-yl)-2-oxabutan-4-oic acid		Goat milk, kidney,[c] and liver Poultry liver [c]
Metabolite 12 1-(6-Propyl-1,3-dibenzodioxol-5-yl)-2,5-dioxaheptan-7-ol		Goat milk,[c] kidney, and liver[b] Poultry egg yolk [c] and liver [b]

Common Name Chemical Name	Structure	Substrate [a]
Metabolite 13 4-[[2-(Hydroxymethoxy)ethoxy]-methyl]-5-propyl-1,2-benzenediol		Goat kidney [b] and liver Poultry egg yolk,[c] liver, and skin [c]
Metabolite 14 1-(6-Propyl-1,3-benzodioxol-5-yl)-2,5-dioxaheptan-7-oic acid		Goat milk, kidney, and liver Poultry egg yolk, liver, thigh muscle,[c] and skin [c]

[a] Compound was identified in both oral and dermal dosing studies unless otherwise indicated.
[b] Only identified in the dermal dosing study.
[c] Only identified in the oral dosing study.

3. Residue Chemistry

Use Information. According to a 12/3/03 search of the Agency's Pesticide Product Label System (PPLS) database, there are 1,647 active products containing PBO as an active ingredient. These products include manufacturing-use and end-use products. On 2/12/03, the Task Force II submitted to the Agency a copy of the master label for PBO which contains all food and non-food sites they wish to retain and support for reregistration. The information contained in the master label was compiled by the Task Force II from hundreds of product labels that contain PBO. PBO may be applied to most agricultural crops preharvest at 0.5 lb ai/application with a maximum number of applications at 10 and a 0 day PHI assumed. PBO may be applied postharvest to vegetables, fruits, and nuts at 0.1 lb ai/1000 ft^2 and to stored grain/seed at 0.027 - 0.057 oz ai/cwt. There are direct treatments of livestock of a 10 % dust or a 0.1 % solution and livestock premise sprays at 0.6 lb ai/1000 ft^2. PBO may be used in eating establishments at 0.56 lb ai/1000 ft^2 and for mosquito abatement at 0.025 - 0.08 lb ai/A.

TABLE 4. Physicochemical Properties of Piperonyl Butoxide		
Parameter	Value	Reference
Boiling point	202-204 °C at 1.9 mm/Hg	D207185, 1/27/99, T. Morton
	180 °C at 1.0 mm/Hg	2002 Farm Chemicals Handbook
pH	Not applicable because the TGAI has low solubility in water	
Density, bulk density, or specific gravity	1.059 g/mL at 20°C	D172854, 11/30/92, A. Aikens
Water solubility	14.34 µg/mL at 25 °C	RD Memorandum, 12/31/90 (cited under D207185, 1/27/99, T. Morton)
Solvent solubility	Completely miscible (95% solution) in acetone, methanol, petroleum distillate, petroleum ether, methylene chloride, and isooctane	D207185, 1/27/99, T. Morton
Vapor pressure	$<1 \times 10^{-7}$ mm Hg at 25 °C (extrapolated from 1.59×10^{-7} mm Hg at 60 °C	D172854, 11/30/92, A. Aikens
Dissociation constant, pK_a	Not applicable because the TGAI has low solubility in water	
Octanol/water partition coefficient	4.51×10^4	RD Memorandum, 12/31/90 (cited under D207185, 1/27/99, T. Morton)
	$\log K_{ow} = 4.95$	D172854, 11/30/92, A. Aikens
UV/visible absorption spectrum	Not available	

Methods. The submitted radiovalidation data demonstrate that the enforcement method is capable of adequately recovering PBO *per se* and its metabolites after acid hydrolysis to a common moiety from lettuce.

Comparison of residues detected in three ^{14}C-treated lettuce samples using the proposed enforcement method and the metabolism method.

	Residues (ppm)		
Analytical Method	PBO	Metabolite A-E	Acid Hydrolyzed Metabolites
Metabolism	2.6, 2.1, 0.8	4.3, 5.8, 2.6	5.2, 4.8, 1.9
Enforcement	2.7, 1.8, 0.7	NA [b]	5.4, 5.3, 1.8

Results.

Lettuce (MRIDs 43227703 and 43832901)

Lettuce plants (approximately one month post-seeding) growing in planter boxes were treated five times, at 10 day intervals, with [^{14}C]PBO labeled at the alpha carbon in the polyether side chain at 0.5 lb ai/A/application for a total rate of 2.5 lb ai/A (1x the maximum proposed seasonal rate for leafy vegetables). Treated lettuce plants were harvested 0 and 10 days following the final application of the test substance.

The total radioactive residues (TRR) were 36.6 and 25.8 ppm in treated lettuce samples collected at Days 0 and 10, respectively. Radioactive residues in/on treated samples were adequately extracted with organic solvents, the extract was partitioned, and the resulting fractions were cleaned up through a C-18 Sep Pak. The nonextractable residues were subjected to a mild acid hydrolysis. All fractions with significant radioactivity were analyzed by chromatographic techniques (HPLC and LC/MS).

In Day-10 lettuce samples, 86% of the TRR was extractable. The parent, PBO, accounted for 24.6% of the TRR; see Table 5. Six metabolites (Metabolites A, B1, B2, C, D, and E) were additionally identified and collectively accounted for a total of ~20% of the TRR. HPLC fluorescence and LC/MS analyses indicated that the detected metabolites contain the PBO double ring structure with sugars or other polar moieties conjugated to the ether side chain.

Table 5. Summary of characterization/identification of residues in lettuce harvested 10 days following the last of five applications of [^{14}C]PBO at 0.5 lb ai/A/application (1X) for a total of 2.5 lb ai/A .

Component	PPM	% TRR
Identified		
PBO	6.36	24.6
Metabolite A	2.2	8.2
Metabolite B	0.6	2.3
Metabolite C	1.8	7.0
Metabolite D	0.5	1.9
Metabolite E	0.2	0.8
Total Identified	11.4	44.2
Characterized		
Polar	2.0	7.7
Non-Polar	--	--
Unknowns (Uncharacterized)	8.5	33.0*
Solids	0.8	3.0
Recovery	23.0	89.0

*Reflects multiple residues none of which exceed 10% of the TRR.

Cotton (MRIDs 43227702, 43832903, and 44082001)

Cotton plants growing in planter boxes were treated six times with a foliar spray of [^{14}C]PBO labeled at the alpha carbon in the polyether side chain at 0.5 lb ai/A/application (1X) for a total rate of 3 lb ai/A (0.6x the maximum proposed seasonal rate). The initial dose was given one month after planting. The interval between applications was 15 days. Because of slow plant maturation, a sixth application was made 2.5 months after the fifth application. Treated leaves were harvested five weeks after the fifth application. Cotton bolls and remaining leaves were harvested 16 days after the last (sixth) dose and manually separated into hulls, seeds, and lint.

The TRRs in treated cotton matrices were as follows: leaves, 142 ppm; hulls, 7.14 ppm; seeds, 0.414 ppm; and lint, 0.528 ppm. Radioactive residues in/on cotton matrices were adequately extracted with organic solvents, the extract was partitioned, and the resulting fractions were cleaned up through a C-18 Sep Pak. Approximately 57-96% of the TRR was extractable from cotton seeds, leaves, lint, and hulls. The nonextractable residues were subjected to a mild acid hydrolysis. All fractions with significant radioactivity were analyzed by HPLC. PBO was detected in the four cotton matrices (8.9-20.8% TRR); no other component was identified. A summary of identified/characterized residues from the cotton metabolism study is presented in Table 6.

Table 6. Summary of characterization/identification of residues in cotton matrices treated with [^{14}C]PBO for a total of 2.5-3.0 lb ai/A.

Component	Seeds		Lint		Hulls		Leaves	
Identified	% TRR	PPM	% TRR	PPM	% TRR	PPM	% TRR	PPM
PBO	20.8	0.086	8.9	0.047	17.3	1.23	18.5	26.3
Total Identified	20.8	0.086	8.9	0.047	17.3	1.23	18.5	26.3
Characterized								
Polar	20.5	0.085	36.2	0.19	23.7	1.69	18.4	26.1
Non-Polar	--	--	--	--	--	--	--	--
Unknowns	55.0	0.21	22.4	0.118	15.9	1.14	41.5	59.1
Solids	21.6	0.089	28.0	0.148	32.5	2.26	25.9	36.9
Recovery	118	0.47	95.5	0.50	89.4	6.32	104	148

The original cotton metabolism submission (MRID 43227702) was initially deemed inadequate but upgradeable. To upgrade the study, the Agency requested further identification and characterization of residues in: (i) cotton seed fraction ACN extract (20.5% TRR; 0.085 ppm) and aqueous extract (50.2% TRR; 0.208 ppm); (ii) cotton hull fraction ACN extract (20.4% TRR; 1.46 ppm); (iii) cotton leaves fraction ACN/MeOH extract (24.4% TRR; 34.7 ppm); (iv) cotton lint fraction MeOH extract (36.2% TRR; 0.191 ppm); and (v) nonextractable residues from any one of the four cotton matrices (seed, lint, hull, and leaves) that contain between 22-33% of the TRR.

The cotton study has subsequently been upgraded to acceptable status following re-analysis of the above cotton fractions/matrices. Upon further investigation of the requested *seed* fractions, the registrant concluded that the fractions contained only highly polar and highly degraded

residues. Likely metabolites are conjugated degradates in which one or both side chains have been oxidized to alcohols or acids, and the dioxole ring has opened to form a catechol. Hydroxymethyl disafrole precursors were not present in the nonextractable residues (PES 4 fraction) of seed which was subjected to mild acid hydrolysis. The acid hydrolysis would have hydrolyzed any precursors to hydroxymethyl disafrole (retained the dioxole ring, propyl sidechain, and benzyl ether) and would have been observed.

Upon further investigation of the requested *hull* extract, the registrant concluded that the residue present might be the substituted benzoic acid derivative of piperonyl butoxide or a conjugate of the benzoic acid. Upon further investigation of the requested *leaf* extract, the registrant concluded that Leaf Component A can be characterized as a moderately polar compound that retained the ether side chain, either oxidized and/or truncated, and also retained the intact dioxole ring, phenyl ring, and propyl side chain. It may have been present as a conjugate, and the conjugating species could possibly include sulfate or glucuronic acid.

Upon further investigation of the requested *lint* extract, the registrant concluded that the extract contained only piperonyl butoxide and polar residues; however, no quantitative data were provided. Upon further investigation of nonextractable residues associated with cotton *hulls*, the released radioactivity detected multiple components. None of the unidentified components exceeded 6.4% of the hull TRR.

Potato (MRIDs 43227701, 43832902, 43946801, and 43946802)

Potato plants growing in planter boxes were treated 4 times, at 15 day intervals, with [^{14}C]PBO labeled at the alpha carbon in the polyether side chain at 0.5 lb ai/A/application for a total rate of 2 lb ai/A (0.4x the maximum proposed seasonal rate for root and tuber vegetables). The initial application was approximately 1.5 months post-planting. Potato tubers and foliage were harvested 8 days after the last dose.

The TRRs in treated potato foliage and tubers were 616.9 and 0.473 ppm, respectively. Radioactive residues in/on treated samples were adequately extracted with organic solvents and the extract was partitioned. The nonextractable residues were subjected to mild acid and base hydrolysis. All fractions with significant radioactivity were analyzed by HPLC.

In tubers, approximately 82% of the TRR was extractable, and a substantial amount of the residue (52% TRR) was aqueous soluble. PBO *per se* was not detected. Several unknowns, each accounting for ≤3.8% of the TRR, accounted for approximately 31% of the residue. In potato foliage, PBO accounted for 39.1% of the TRR, and one unknown accounted for 31.6% of the radioactivity. Several aqueous soluble components were detected, but each accounted for ≤3% of the TRR. The registrant stated that comparison of potato tuber and foliage HPLC profiles revealed no component peaks with similar retention times above the limit of quantitation (0.011 ppm). The data indicate that there is limited translocation of PBO from leaves to tubers. A summary of identified/characterized residues from the potato metabolism study is presented below in Table 7.

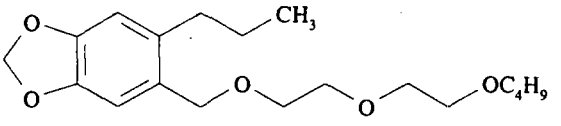
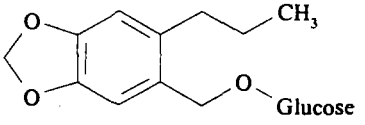
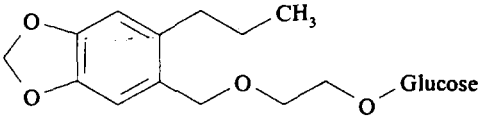
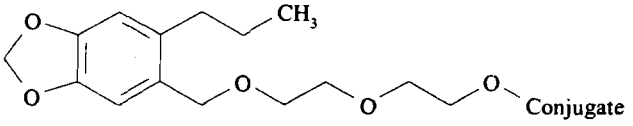
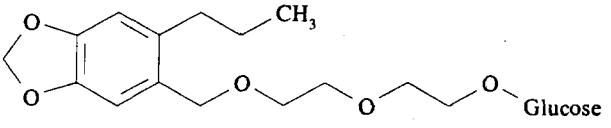
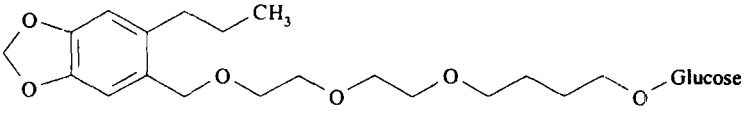
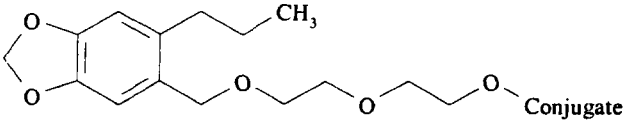
Table 7. Summary of characterization/identification of residues in potato matrices treated with [^{14}C]PBO for a total of 2.0 lb ai/A.				
Component	Tubers		Leaves	
Identified	% TRR	PPM	% TRR	PPM
PBO	ND	ND	39.1	240.9
Total Identified	0.0	0.0	39.1	240.9
Characterized				
Polar	--	--	--	--
Non-Polar	--	--	--	--
Unknown (Uncharacterized)	82.7	0.39	47.2	291.4
Solids	7.4	0.035	36.21	223.4
Recovery	90.1	0.425	122.5	755.7

The original potato metabolism submission (MRID 43227701) was initially deemed inadequate but upgradeable. To upgrade the study, the Agency requested further identification and characterization of residues in: (i) two aqueous fractions of tubers accounting for 16.5% (0.078 ppm) and 35.6% (0.168 ppm) of the TRR; and (ii) the petroleum ether extract of the leaves accounting for 31.6% (195.2 ppm) of the TRR.

The potato study has subsequently been upgraded to acceptable status following re-analysis of the tuber's aqueous fractions. Upon further investigation of Aqueous A fraction, the bulk of radioactivity was characterized to be comprised of 15 or more components each present at <2% TRR. The results of additional work-up on Aqueous B Fraction also resolved 15 compounds each present at <2% TRR. No further identification/characterization of radioactivity in Aqueous Fractions A and B was performed.

No additional work-up on potato leaves was performed because potato foliage is not considered a significant food/feed item and is not listed in Table 1 of GLN 860.1000. The molecular structures of PBO and its plant metabolites are presented in Table 8.

Table 8. Molecular structures of PBO and its plant metabolites.

Chemical/Common Name	Structure
Piperonyl Butoxide/PBO	
Metabolite A Glucose conjugate of 6-propyl-1,3-benzodioxol-5-methanol	
Metabolite B1 Glucose conjugate of 1-(6-propyl-1,3-dibenzodioxol-5-yl)-2-oxabutan-4-ol	
Metabolite B2 Conjugate of 1-(6-propyl-1,3-dibenzodioxol-5-yl)-2,5-dioxaheptan-7-ol	 Conjugate is larger and more polar than glucose.
Metabolite C Glucose conjugate of 1-(6-propyl-1,3-dibenzodioxol-5-yl)-2,5-dioxaheptan-7-ol	
Metabolite D Glucose conjugate of 1-(6-propyl-1,3-dibenzodioxol-5-yl)-2,5,8-trioxadodecan-12-ol	
Metabolite E Conjugate of 1-(6-propyl-1,3-dibenzodioxol-5-yl)-2,5-dioxaheptan-7-ol	 Conjugate is smaller and less polar than glucose and is likely to contain nitrogen.

Summary of Metabolism in Livestock (Goat - MRID 43643201; Poultry - MRID 43712601).

The qualitative nature of the residue in ruminants and poultry, resulting from dermal treatments, is adequately understood. However, the nature of the residue in ruminants and poultry, resulting from oral treatments, is only partially understood. The oral metabolism studies may be upgraded to acceptable status pending further characterization/identification of radioactive residues in certain matrices/fractions and radiovalidation of the enforcement or data-collection method using samples from either the dermal or oral studies.

The reviewed animal metabolism studies confirm that residues of PBO will be incurred in eggs, milk, and ruminant and poultry tissues following dermal and oral treatments at the dose levels utilized in these studies. The Agency will re-assess the adequacy of the tolerance expression for animal commodities when the additional requested data have been submitted and reviewed. Brief summaries of the available animal metabolism studies are presented below.

Ruminants (MRID 43643201)

The metabolism of PBO in lactating goats following multiple oral or dermal administrations was separately investigated. The test substance, [^{14}C]PBO, was uniformly labeled in the phenyl ring. During the testing period, the goats were fed commercial goat feed and alfalfa cubes and allowed water *ad libitum*. Milk was collected at 12-hour intervals after the start of the dosing period until termination. The goats were sacrificed 22 hours after the last dose, and the following samples were collected: omental fat with peripheral fat (adjacent to the omentum), muscle (leg plus loin), heart, liver, and kidney.

For the dermal study, one lactating goat received [^{14}C]PBO by application to the skin as a 10% solution in methanol for five days. According to the registrant's calculations, the goat administered [^{14}C]PBO by dermal application, received an amount of PBO that represented a dose equivalent to 0.23 mg per kg of body weight, or to 6.85 ppm if calculated based on the goat's feed intake. The dose solution to be administered was added in a dropwise manner onto a shaved area of the goat's back and spread evenly over the test area with a pipette.

The TRR in dermally treated samples were: 0.196 ppm in fat, 0.149 ppm in liver, 0.113 ppm in kidney, 0.040 ppm in composite milk, and 0.023 ppm in composite muscle. These results indicate that the distribution of radioactivity is affected by the route of administration. In the dermal treatment study, fat contained the highest level of radioactivity.

The majority of ^{14}C-residues were characterized/identified in leg muscle (~90% TRR) and fat (~87% TRR) of dermally treated goats. Although only ~44%, ~48%, and ~57% of the TRR in liver, kidney, and milk, respectively, were characterized/identified, no additional analytical work was carried out for these tissues since the remaining radioactive residues consisted of non-extractable residues comprising 0.001-0.033 ppm, unidentified chromatographic peaks comprising <0.001-0.005 ppm, and lost/unaccounted radioactivity comprising 0.015-0.054 ppm.

The parent, PBO, was identified in all matrices of dermally treated goats. PBO was the major residue in milk (31.02% TRR), leg muscle (61.84% TRR), fat (79.22% TRR), and liver (4.76% TRR). Four metabolites (Metabolites 11, 12, 13, and 14) were additionally identified. A summary of radioactive residues characterized/identified in the milk and tissues of dermally treated goat is presented in Table 9.

Table 9. Summary of radioactive residues characterized/identified in milk and tissues from a lactating goat dermally treated with uniformly ring-labeled [^{14}C]PBO as a 10% solution for five days.

Metabolite/Fraction	Whole Milk (TRR=0.040 ppm)		Fat (TRR=0.196 ppm)		Kidney (TRR=0.113 ppm)		Liver (TRR=0.149 ppm)		Leg Muscle (TRR=0.023 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified										
PBO	31.02	0.012	79.22	0.155	6.46	0.007	4.76	0.007	61.84	0.014
Metabolite 11	1.86	0.001	--	--	--	--	4.12	0.006	--	--
Metabolite 12	--	--	--	--	9.13	0.010	6.09	0.010	--	--
Metabolite 13	--	--	--	--	2.83	0.003	12.25	0.018	--	--
Metabolite 14	1.48	0.001	--	--	5.73	0.006	9.22	0.014	--	--
Total Identified	**34.36**	**0.013**	**79.22**	**0.155**	**24.15**	**0.027**	**36.44**	**0.054**	**61.84**	**0.014**
Characterized										
Metabolite A	--	--	--	--	1.33	0.002	--	--	--	--
Metabolite 3	1.54	0.001	--	--	2.90	0.003	--	--	--	--
Metabolite 4	--	--	--	--	1.23	0.001	--	--	--	--
Metabolite 5	7.36	0.003	--	--	2.33	0.003	--	--	--	--
Metabolite 6	--	--	--	--	1.26	0.001	--	--	--	--
Metabolite 6A	0.46	<0.001	--	--	--	--	--	--	--	--
Metabolite 6B	1.50	0.001	--	--	2.37	0.003	--	--	--	--
Metabolite 7A	--	--	--	--	4.43	0.005	--	--	--	--
Metabolite 9	--	--	--	--	1.80	0.002	--	--	--	--
Metabolite 9A	0.73	<0.001	--	--	--	--	--	--	--	--
Metabolite 15	--	--	--	--	--	--	0.88	0.001	--	--
Metabolite 16	--	--	--	--	--	--	0.65	0.001	--	--
R_t = 3.33 min	--	--	--	--	0.78	0.001	--	--	--	--
R_t = 3.5 min	--	--	--	--	--	--	--	--	5.89	0.001
R_t = 4.5 min	--	--	--	--	--	--	--	--	7.29	0.002
Sep-Pak Aqueous	11.06	0.004	0.05	<0.001	2.78	0.003	0.73	0.001	14.66	0.003
Sep-Pak Acetone	--	--	0.81	0.002	--	--	1.53	0.002	--	--
Sep-Pak Ethyl Acetate	--	--	--	--	0.58	0.001	0.30	<0.001	--	--
MeOH	--	--	7.21	0.014	1.98	0.002	3.68	0.005	--	--
Total Identified/ Characterized	**57.01**	**0.023**	**87.29**	**0.171**	**47.92**	**0.054**	**44.21**	**0.066**	**89.68**	**0.021**
Non-Extractable	2.76	0.001	5.23	0.010	4.57	0.005	21.92	0.033	16.21	0.004
Loss/Unaccounted	40.23	0.016	7.48	0.015	47.51	0.054	33.87	0.050	--	--

For the oral study, two lactating goats received [^{14}C]PBO by gavage using a balling gun at nominal dose levels equivalent to 10 and 100 ppm of daily feed intake for five consecutive days. The feeding levels are ~0.1x and 1x the tentative maximum theoretical dietary exposure of dairy cattle to PBO residues.

Following oral administration of the test substance at 100 ppm in the diet, the TRR were: 2.007 ppm in liver, 0.398 ppm in kidney, 0.317 ppm in composite milk, 0.234 ppm in fat, and 0.008 ppm in composite muscle. No further work was conducted with muscle tissues because of low radioactivity levels. In the orally dosed goats, the highest level of radioactivity occurred in liver.

The majority of ^{14}C- residues were adequately characterized/identified in fat (~82% TRR). However, only ~36% of TRR in liver, ~52% of TRR in milk, and ~53% of TRR in kidney was characterized or identified; this is a data gap. To upgrade this study, the registrant was requested to conduct additional analytical work on the aqueous fraction of liver (10.04% of TRR, 0.201 ppm) remaining after acid hydrolysis of non-extractable residues, and on the remaining non-extractable residues (31.46% of TRR, 0.631 ppm) of liver. The registrant was also requested to conduct additional work with milk and kidney to identify metabolites such that a greater percentage of the TRR is identified. For liver, milk, and kidney, an explanation for the ~32%, 45%, and 39% of TRR, respectively, that was lost or unaccounted for in the isolated fractions or HPLC peaks must be provided. A summary of radioactive residues characterized/identified in the milk and tissues of orally treated goat is presented in Table 10.

Table 10. Summary of radioactive residues characterized/identified in milk and tissues from a lactating goat orally dosed with uniformly ring-labeled [^{14}C]PBO at 100 ppm in the diet for five days.

Metabolite/Fraction	Whole Milk (TRR=0.317 ppm)		Fat (TRR=0.234 ppm)		Kidney (TRR=0.399 ppm)		Liver (TRR=2.007 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified								
PBO	1.81	0.006	55.31	0.129	2.30	0.010	5.69	0.115
Metabolite 11	1.44	0.005	--	--	5.93	0.024	2.01	0.040
Metabolite 12	0.68	0.002	--	--	5.79	0.023	--	--
Metabolite 13	--		--	--	--	--	6.75	0.136
Metabolite 14	4.93	0.016	--	--	11.46	0.045	3.71	0.075
Total Identified	**8.86**	**0.028**	**55.31**	**0.129**	**25.48**	**0.102**	**18.16**	**0.364**
Characterized								
Metabolite B	--		--	--	0.17	0.001	--	--
Metabolite C	1.89	0.006	--	--	--	--	--	--
Metabolite 1A	2.33	0.007	--	--	--	--	--	--
Metabolite 1B	0.67	0.002	--	--	--	--	0.84	0.017
Metabolite 2	5.27	0.017	--	--	--	--	--	--
Metabolite 2A	0.72	0.002	--	--	--	--	--	--
Metabolite 3	3.34	0.011	--	--	2.01	0.009	--	--
Metabolite 4	1.39	0.004	--	--	2.03	0.008	--	--
Metabolite 5	12.95	0.041	--	--	2.73	0.011	--	--
Metabolite 6	1.37	0.004	--	--	4.14	0.017	--	--
Metabolite 6A	0.91	0.003	--	--	6.12	0.024	0.67	0.013
Metabolite 7A	1.00	0.003	--	--	3.67	0.015	0.91	0.018
Metabolite 7B	--		--	--	--	--	0.69	0.014
Metabolite 8	1.50	0.005	--	--	--	--	--	--
Metabolite 8A	2.24	0.007	--	--	1.51	0.006	--	--
Metabolite 9	5.23	0.017	--	--	--	--	--	--
Metabolite 15	0.16	0.001	20.03	0.047	--	--	1.40	0.028
Metabolite 16	0.22	0.001	--	--	--	--	1.88	0.038
Metabolite 19	2.30	0.007	--	--	--	--	--	--
Sep-Pak Aqueous	--	--	--	--	--	--	0.05	0.001
Aqueous	--		6.70	0.016	5.54	0.022	10.04	0.201
Sep-Pak Acetone	--		--	--	--	--	1.57	0.032
Total Identified/ Characterized	**52.35**	**0.166**	**82.04**	**0.192**	**53.40**	**0.213**	**36.21**	**0.727**
Non-Extractable	2.73	0.009	ND	ND	7.91	0.032	31.46	0.631
Loss/Unaccounted	44.92	0.142	17.96	0.042	38.69	0.154	32.33	0.649

Poultry

The metabolism of PBO in poultry following multiple oral or dermal administrations was separately investigated. The test substance, [^{14}C]PBO, was uniformly labeled in the phenyl ring. During the testing period, all hens were fed Purina Lab Cage layer chow and allowed water *ad libitum*. Eggs were collected once daily from each hen prior to daily dosing, and the collected eggs were separated into yolk and white fractions. The hens were sacrificed 20 to 23 hours after administration of the last dose. Liver, fat, muscle (breast and thigh), skin (from the application site and an untreated site), and kidney were collected at sacrifice.

For the dermal study, ten hens received a dermal application of a dosing solution containing [^{14}C]PBO at a nominal dose level equivalent to 10 ppm of the daily feed intake. The test hens were anesthetized with an intravenous injection of ketamine 24 hours prior to dosing. The dose solution to be administered was added in a dropwise manner using a pipette.

The TRR in dermally treated samples were: 0.347 ppm in fat, 0.158 ppm in liver, 0.083 ppm in skin, 0.007 ppm in thigh muscle, and 0.003 ppm in breast muscle. Unlike the goat study, the distribution of radioactivity in poultry tissues following dermal treatment was similar to that following oral treatment. The TRR in composite egg whites and yolks were <0.001-0.013 ppm and <0.001-0.093 ppm, respectively, over the course of dosing. Residues in egg whites appeared to plateau on the second day of dosing while residues in egg yolks increased each day of dosing. No further work was conducted with thigh and breast muscle because of low radioactivity levels.

The majority of ^{14}C-residues were sufficiently characterized/identified in fat (~79% TRR), skin (~79% TRR), egg white (~84% TRR), and egg yolk (~79% TRR) of dermally treated hens. Although only ~38% of the TRR in liver were characterized/identified, no additional analytical work was conducted for liver because the remaining radioactivity consisted of uncharacterized extracts each <0.03 ppm, non-extractable residues at 0.059 ppm, and unidentified chromatographic peaks each ≤0.002 ppm.

The parent, PBO, was identified in all matrices of dermally dosed hens. PBO was the major residue in fat (79.09% TRR), skin (72.16% TRR), egg white (75.51% TRR), egg yolk (62.13% TRR), and liver (8.38% TRR). Several metabolites were also identified. A summary of radioactive residues characterized/identified in the eggs and tissues of dermally treated hens is presented in Table 11.

Table 11. Summary of radioactive residues characterized/identified in eggs and tissues from laying hens dermally treated with uniformly ring-labeled [^{14}C]PBO at a nominal dose level equivalent to 10 ppm of the daily feed intake for five days.

Metabolite/Fraction	Egg Yolk (TRR=0.093 ppm)		Egg White (TRR=0.013 ppm)		Fat (TRR=0.347 ppm)		Liver (TRR=0.158 ppm)		Skin (TRR=0.083 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified										
PBO	62.13	0.058	75.51	0.010	79.09	0.274	8.38	0.013	72.16	0.060
Metabolite 12	--	--	--	--	--	--	1.51	0.002	--	--
Metabolite 13	--	--	--	--	--	--	0.94	0.001	--	--
Metabolite 14	10.15	0.009	--	--	--	--	5.35	0.008	--	--
Total Identified	**72.28**	**0.067**	**75.71**	**0.010**	**79.09**	**0.274**	**16.18**	**0.026**	**72.16**	**0.060**
Characterized										
Metabolite 3	2.40	0.002	--	--	--	--	0.61	0.001	--	--
Metabolite 5	1.27	0.001	--	--	--	--	0.62	0.001	--	--
Metabolite 8	--	--	--	--	--	--	0.77	0.001	--	--
Metabolite 9A	0.67	0.001	--	--	--	--	1.12	0.002	--	--
Metabolite 15	--	--	--	--	--	--	0.62	0.001	--	--
R_t = 9.63 min	2.12	0.002	--	--	--	--	--	--	--	--
Sep-Pak Aqueous	0.03	<0.001	0.83	<0.001	0.25	0.001	3.78	0.006	--	--
Sep-Pak Acetone	--	--	7.64	0.001	--	--	--	--	--	--
Ethyl Acetate	--	--	--	--	--	--	0.78	0.001	--	--
Aqueous	--	--	--	--	--	--	17.19	0.028	6.35	0.005
Total Identified/Characterized	**78.77**	**0.073**	**84.18**	**0.011**	**79.34**	**0.275**	**37.93**	**0.060**	**78.51**	**0.065**
Non-Extractable	31.99	0.030	5.64	0.001	5.76	0.020	37.44	0.059	3.58	0.003
Loss/Unaccounted	--	--	10.18	0.001	14.90	0.052	24.63	0.039	17.91	0.015

For the oral dose study, ten hens received [^{14}C]PBO by gavage at nominal dose levels equivalent to 10 and 100 ppm of daily feed intake for five consecutive days. The feeding levels are 50x and 500x the tentative maximum theoretical dietary exposure of poultry to PBO residues.

Following oral administration of the test substance to laying hens at 100 ppm in the diet, the TRR were: 5.488 ppm in fat, 1.619 ppm in liver, 0.982 ppm in skin, 0.158 ppm in thigh muscle, and 0.032 ppm in breast muscle. The TRR in composite egg whites and yolks were 0.052-0.629 ppm and 0.004-1.933 ppm, respectively. The total radioactivity in composite egg whites and yolks generally increased during the course of dosing.

The majority of ^{14}C-residues were sufficiently characterized/identified in fat (~78% TRR), skin (~76% TRR), egg white (>100% TRR), egg yolk (~83% TRR), and thigh muscle (~86% TRR) of orally dosed hens. However, only ~50% of TRR was characterized/identified in liver which is a study deficiency. To upgrade this study, the registrant was requested to conduct additional analytical work on the aqueous fraction of liver (19.97% of TRR, 0.323 ppm) resulting after hydrolysis of the nonextractable residues, and on the remaining non-extractable residues of liver (46.61% of TRR, 0.755 ppm).

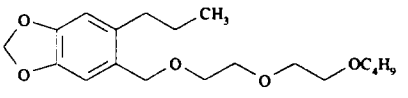
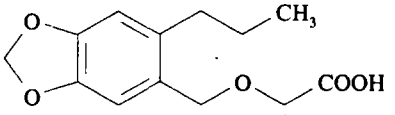
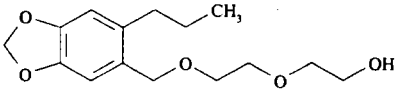
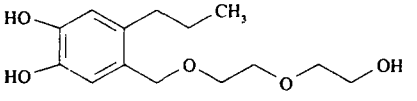
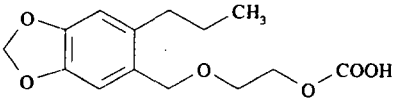
The parent, PBO, was identified in all matrices of orally dosed hens except liver. PBO was the major residue in fat (78.27% TRR), skin (45.31% TRR), egg white (>100% TRR), egg yolk (61.11% TRR), and thigh muscle (72.80% TRR). Four additional metabolites were identified in tissues. A summary of radioactive residues characterized/identified in the eggs and tissues of orally treated hens is presented in Table 12.

The molecular structures of PBO and its animal metabolites are presented in Table 13. The proposed metabolic pathway of PBO in lactating goats and laying hens is depicted in Figure 1.

Table 12. Summary of radioactive residues characterized/identified in eggs and tissues from laying hens orally treated with uniformly ring-labeled [^{14}C]PBO at a nominal dose level of 100 ppm of the daily feed intake for five days.

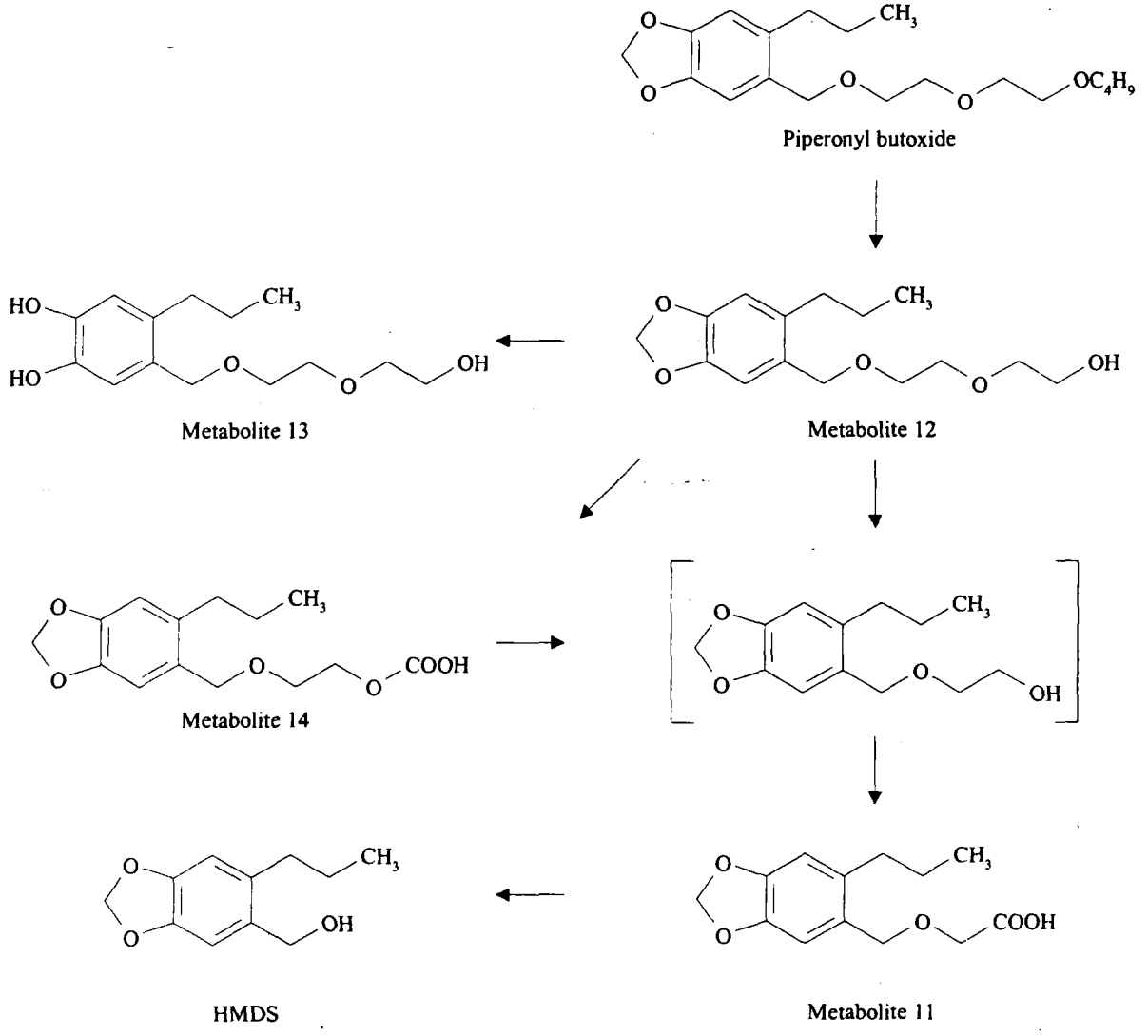
Metabolite/Fraction	Egg Yolk (TRR=1.933 ppm)		Egg White (TRR=0.442 ppm)		Fat (TRR=5.488 ppm)		Liver (TRR=1.619 ppm)		Thigh Muscle (TRR=0.158 ppm)		Skin (TRR=0.982 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified												
PBO	61.11	1.181	100.58	0.445	78.27	4.295	--	--	72.80	0.115	45.31	0.445
Metabolite 11	--	--	--	--	--	--	3.10	0.050	--	--	--	--
Metabolite 12	0.74	0.014	--	--	--	--	--	--	--	--	--	--
Metabolite 13	0.75	0.015	--	--	--	--	3.52	0.057	--	--	12.52	0.123
Metabolite 14	9.31	0.180	--	--	--	--	9.02	0.146	0.80	0.001	13.19	0.130
Total Identified	**71.91**	**1.390**	**100.58**	**0.445**	**78.27**	**4.295**	**15.64**	**0.253**	**73.60**	**0.116**	**71.02**	**0.697**
Characterized												
Metabolite AA	--	--	--	--	--	--	--	--	0.81	0.001	--	--
Metabolite A	--	--	--	--	--	--	1.50	0.024	--	--	--	--
Metabolite 3	--	--	--	--	--	--	1.86	0.030	--	--	--	--
Metabolite 5	--	--	--	--	--	--	0.44	0.007	--	--	--	--
Metabolite 7A	0.43	0.008	--	--	--	--	--	--	--	--	--	--
Metabolite 8	--	--	--	--	--	--	1.88	0.030	--	--	--	--
Metabolite 9A	--	--	--	--	--	--	0.72	0.012	--	--	--	--
Metabolite 15	--	--	--	--	--	--	1.16	0.019	--	--	--	--
Metabolite 18	--	--	--	--	--	--	--	--	1.87	0.003	--	--
R_t = 3.5 min	--	--	--	--	--	--	--	--	2.67	0.004	--	--
R_t = 4.5 min	--	--	--	--	--	--	--	--	7.23	0.011	--	--
R_t = 10.18 min	3.16	0.061	--	--	--	--	--	--	--	--	--	--
Petroleum Ether	--	--	--	--	--	--	4.96	0.080	--	--	--	--
Sep-Pak Aqueous	0.35	0.007	0.18	0.001	0.01	0.001	1.05	0.017	--	--	4.53	0.044
Sep-Pak Acetone	--	--	5.10	0.023	--	--	--	--	--	--	--	--
Ethyl Acetate	1.43	0.028	--	--	--	--	0.54	0.009	--	--	--	--
Aqueous	5.58	0.108	--	--	--	--	19.97	0.323	--	--	--	--
Total Identified/Characterized	**82.86**	**1.602**	**105.86**	**0.468**	**78.28**	**4.296**	**49.72**	**0.805**	**86.18**	**0.136**	**75.55**	**0.742**
Non-Extractable	17.69	0.342	2.44	0.011	5.12	0.281	46.61	0.755	8.09	0.013	6.75	0.066
Loss/Unaccounted	--	--	--	--	16.60	0.911	3.67	0.059	5.73	0.009	17.70	0.174

Table 13. Molecular structures of PBO and its metabolites in lactating goats and laying hens.

Common Name Chemical Name	Structure	Substrate [a]
Piperonyl butoxide (Butylcarbityl)(6-propyl piperonyl)ether		Goat milk, fat, kidney, liver, leg muscle,[b] and loin muscle [b] Poultry egg yolk, egg white, fat, liver,[b] thigh muscle,[c] and skin
Metabolite 11 1-(6-Propyl-1,3-benzodioxol-5-yl)-2-oxabutan-4-oic acid		Goat milk, kidney,[c] and liver Poultry liver [c]
Metabolite 12 (Metabolite M5 in the Rat Metabolism study) 1-(6-Propyl-1,3-dibenzodioxol-5-yl)-2,5-dioxaheptan-7-ol		Goat milk,[c] kidney, and liver [b] Poultry egg yolk [c] and liver [b]
Metabolite 13 4-[[2-(Hydroxymethoxy)ethoxy]-methyl]-5-propyl-1,2-benzenediol		Goat kidney [b] and liver Poultry egg yolk,[c] liver, and skin [c]
Metabolite 14 (Metabolite M7 in the Rat Metabolism study) 1-(6-Propyl-1,3-benzodioxol-5-yl)-2,5-dioxaheptan-7-oic acid		Goat milk, kidney, and liver Poultry egg yolk, liver, thigh muscle,[c] and skin [c]

[a] Compound was identified in both oral and dermal dosing studies unless otherwise indicated.
[b] Only identified in the dermal dosing study.
[c] Only identified in the oral dosing study.

Figure 3.4. Proposed metabolic pathway of piperonyl butoxide in lactating goats and laying hens.



Summary of Metabolism in Confined Rotational Crops.

A confined rotational crop study (OPPTS 860.1850) is required to determine the nature and amount of pesticide residue uptake in rotational crops as well as appropriate rotational crop restrictions. A field accumulation study in rotational crops (OPPTS 860.1900) is required if the level of the total radioactive residue in the confined rotational crops is equal to or exceeds 0.01 ppm at the desired rotational interval or at 12 months, and once the nature of the residue in the rotational crops is understood.

Summary of Analytical Methods

Plant commodities

The Pesticide Analytical Manual (PAM) Volume II lists a colorimetric method (Method II) for the enforcement of tolerances for residues of PBO *per se* in/on plant commodities. An improved method, HPLC/fluorescence method (EN-CAS Method ENC-14/93 and/or Pharmaco LSR PBO Method) entitled "Analysis of Piperonyl Butoxide and Its Plant Metabolites in Crop Matrices by High Performance Liquid Chromatography", has been proposed to replace the existing colorimetric method. The method can separately determine residues of PBO *per se* and PBO metabolites collectively determined as hydroxymethyl dihydrosafrole (HMDS).

The proposed HPLC/fluorescence method has been subjected to an independent laboratory validation (ILV) using a leafy vegetable (lettuce) and a root and tuber crop (potato) as the matrices. During the ILV, the samples were fortified at 5 ppm and 20 ppm; the samples were not fortified at the limit of quantitation (LOQ; 0.1 ppm). Validation recoveries ranged 93.1% to 106.6% (average=99.6%, N=4) for lettuce samples and 44.5% to 88.9% (N=4) for potato samples. A second analysis set of potato samples was analyzed, and validation recoveries ranged 92.7% to 99.0% (average=96.4%, N=4).

The Agency review (DP Barcode D227456, 1/27/99, T. Morton) concluded that the ILV performed satisfactorily at the 5 and 20 ppm fortification levels. Although, no fortification was made at the LOQ level, HED waived that requirement since the new method is an improvement of the current enforcement method. HED has forwarded the HPLC/fluorescence method to ACL/BEAD (DP Barcode D252542, 1/28/99, T. Morton) for method validation by Agency chemists.

The data-collection method used for the analysis of samples, harvested from recent studies pertaining to magnitude of the residue and storage stability studies, was the same HPLC/fluorescence method. A brief description of the method follows. Residues in plant commodities are extracted twice with acetonitrile (ACN) in the presence of Celite. The extract is concentrated to remove the ACN, a 5% sodium chloride solution is added, and the extract is partitioned twice with petroleum ether. The petroleum ether phases, which contain PBO residues, are combined and an aliquot is concentrated to dryness under a stream of nitrogen. The aqueous phase, which contains residues of PBO metabolites to be determined collectively as HMDS, is stored frozen for later analysis. Residues are dissolved in ACN and injected onto an HPLC equipped with a C-18 column and a fluorescence detector (288 nm). The mobile phase

consists of ACN:water (70:30, v:v). For cotton forage, the mobile phase consisted of ACN:water (65:35, v:v). The limit of quantitation (LOQ) is 0.1 ppm.

The HPLC/fluorescence method was adequately radiovalidated using aged samples from the lettuce metabolism study. The radiovalidation data demonstrate that the data-collection method is capable of adequately recovering PBO *per se* and the metabolites identified in the lettuce metabolism study (Metabolites A through E) after acid hydrolysis to a common moiety (2-propanyl-4,5-methylenedioxobenzyl alcohol).

Animal commodities

Under Section 180.127, PAM Volume II lists several methods for the enforcement of tolerances for residues of PBO *per se* in animal commodities. The gas liquid chromatography methods (Method I and Methods A and B) are preferred over the colorimetric methods (Methods C and D). Using the GLC methods, residues in samples of milk and tissues are extracted with a mixture of ethyl alcohol, ether, and hexane. Saponification with alcoholic KOH removes most of the fatty material. The saponified extract in hexane is chromatographed through a florisil column to separate the piperonyl butoxide from chlorinated hydrocarbon pesticides. The final cleanup is accomplished by thin layer chromatography. Determination is by GLC equipped with flame ionization detector for Methods I and B, and electron capture detector for Method A. The sensitivity of each method is 0.005 ppm.

Tolerances for animal commodities are presently established and expressed in terms of residues of piperonyl butoxide *per se*. If the HED MARC determines that any PBO metabolite(s) identified in the animal metabolism studies is of toxicological concern and should be included in the tolerance expression, then additional animal enforcement methods may be required.

The data-collection method used for the analysis of samples, collected from separate studies reflecting oral, dermal, and premise spray treatments, is a GC/MS/MS (electron impact) method developed at PTRL Europe (PTRL Europe Project/Report No. P 173 G, 9/11/95). A description of the data-collection method, is briefly described below.

Samples of eggs, milk, and tissues are mixed with sodium sulfate and Celite and then extracted twice with acetonitrile (ACN). After centrifugation and filtration, the extract is partitioned with hexane to remove fat. The hexane phase is removed and washed with ACN, and the two ACN phases are combined. The ACN extract is concentrated by rotary evaporation, 1.5% NaCl is added, and the extract is partitioned with hexane. The phases are allowed to separate and the aqueous phase is partitioned with hexane. The hexane phases are combined and dried over sodium sulfate; after the addition of toluene, the extract is concentrated by rotary evaporation. The concentrated extract is then cleaned up on a silica gel column; residues are eluted with toluene:ethyl acetate (9:1, v:v). The eluate is concentrated by rotary evaporation and then evaporated to dryness under nitrogen. Dried extracts are redissolved in toluene and analyzed by GC/MS/MS. Quantitative results are obtained by GC/MS/MS analysis using a DB5 MS column. The GC/MS/MS system uses an electron impact ionization MS detector with collision induced dissociation. The limits of quantitation are 0.01 ppm for eggs and milk, and 0.05 ppm for cattle and poultry tissues.

Multiresidue Methods (MRID).

According to PAM Volume I, piperonyl butoxide *per se* is completely recovered by multiresidue method Protocols A and D. A recent submission (MRID 44604201) confirms that head lettuce gave acceptable recoveries for piperonyl butoxide *per se* when taken through the complete method in Protocol A using 401E1 + C1 + DL2. The Agency will require additional multiresidue methods data if it is determined that additional residues of concern, other than the parent, need to be included in the tolerance expression.

Summary of Magnitude of the Residue Studies – Crops

Table 14. Summary of Residues from the Crop Field Trials with Piperonyl Butoxide.

Crop Matrix	Residues	
	Min. (ppm)	Max. (ppm)
Root and Tuber (supported use = 0.5 lb ai/A total application rate, 10 applications, 0-day PHI)		
Carrot	<0.73	<1.21*
Potato	<0.20	<0.21*
Radish root	<0.27	<0.44*
Radish tops	45	49*
Sugar beet root	<0.20	<0.20*
Sugar beet tops	12	19*
Leafy Vegetables (except Brassica) (supported use = 0.5 lb ai/A total application rate, 10 applications, 0-day PHI)		
Celery	6.49	23*
Head lettuce with wrapper leaves	4.12	6.20*
Head lettuce without wrapper leaves	<0.19	0.64*
Leaf lettuce	19	25*
Spinach	34	43*
Brassica (supported use = 0.5 lb ai/A total application rate, 10-12 applications, 0-day PHI)		
Broccoli	0.63	2.28
Cabbage with wrapper leaves	0.79	6.42
Cabbage without wrapper leaves	<0.05	0.46
Mustard greens	25	38
Legume Vegetables (Succulent and Dried) (supported use = 0.5 lb ai/A total application rate, 10-13 applications, 0-day PHI)		
Succulent bean pods	0.28	2.15
Dry bean seed	<0.10	0.11
Succulent pea pods	0.97	5.14
Dry pea seed	0.10	0.57

Crop Matrix	Residues	
	Min. (ppm)	Max. (ppm)
Foliage of Legume Vegetables (supported use = 0.5 lb ai/A total application rate, 10-13 applications, 0-day PHI)		
Succulent pea vines	23	47
Succulent pea hay	25	153
Dry pea vines	27	97
Dry pea hay	1.20	48
Fruiting Vegetables (supported use = 0.5 lb ai/A total application rate, 10 applications, 0-day PHI)		
Pepper	<0.27	1.49*
Tomato	<0.29	1.11*
Cucurbit Vegetables (supported use = 0.5 lb ai/A total application rate, 10-11 applications, 0-day PHI)		
Cantaloupe	<0.49	<0.93*
Cucumber	<0.19	0.90*
Squash	<0.15	0.40*
Citrus Fruits (supported use = 0.5 lb ai/A total application rate, 10 applications, 0-day PHI)		
Grapefruit	0.27	1.42
Lemon	1.14	3.11
Orange	0.54	1.03
Berries (supported use = 0.5 lb ai/A total application rate, 10 applications, 0-day PHI)		
Blackberry	2.65	2.85
Blueberry	4.19	5.51
Herbs and Spices (supported use = 0.5 lb ai/A total application rate, 10 applications, 0-day PHI)		
Mustard seed	<0.10	2.10
Miscellaneous Commodities (supported use = 0.5 lb ai/A total application rate, 10 applications, 0-day PHI)		
Cottonseed	<0.10	0.21
Cotton forage	11	37
Cranberry	5.62**	8.36**
Grape	15**	19**
Strawberry	2.62**	6.24**

*combined residues of PBO and PBO metabolites

**residue levels were corrected for residue decline

Peanut

A tolerance of 8 ppm has been established for residues of PBO in/on peanut (with shell removed) resulting from postharvest uses [40 CFR §180.127(a)(1)]. The peanut tolerance as well as the tolerances for various raw agricultural commodities resulting from postharvest treatments were approved by the USDA in conjunction with PP#0048. As stated in a memo in PP#0048 (L.L. Ramsey, 7/9/56), no peanut residue data were submitted; the tolerance on peanuts was established based on translation and calculations.

Preharvest uses: The Task Force II is not supporting preharvest uses on peanuts; therefore, no preharvest data are required.

Postharvest uses: Data (MRID 42131904), depicting the magnitude of PBO residues in/on peanuts stored in a warehouse situation, have been submitted and reviewed (DP Barcode D179588, 12/29/92, N. Dodd). The reviewed data are inadequate because they do not reflect the maximum use rates the PBO Task Force II wishes to support for postharvest uses on peanuts. In addition, the submitted data do not reflect actual sampling in accordance with the protocol established and used by FDA in tolerance enforcement.

The peanut study was conducted in 1989 inside an 8,200 cu. ft room at the USDA Stored-Product Insects Research and Development Laboratory (Savannah, GA). Approximately 9 lbs. of farmers stock peanuts were weighed into each of 33 cardboard boxes measuring 1 ft by 1 ft with a height of 0.5 ft. These boxes were set in three rows on the floor, 11 boxes per row, along the length of the room. The study did not specify whether the cardboard boxes containing the peanuts were open or closed.

The test substance was a pressurized spray formulation containing 1.50% active ingredients (0.25% esfenvalerate, 1.25% piperonyl butoxide) and 98.50% inerts. PBO was applied at a rate of 0.1 g ai/1,000 cu. ft per application for 235 days. According to the master label, the maximum PBO use rates which the Task Force II wishes to support are: (i) 0.0027 lb ai/1,000 cu. ft (1.226 g ai/1,000 cu. ft) for space treatments of storage areas where bulk or bagged peanuts may be stored postharvest; and (ii) 0.03 lb ai/1,000 cu. ft (14.98 g ai/1,000 cu. ft) for general space treatments of peanut processing plants. Therefore, the PBO rate used in the study is <1.0x the rates the Task Force II wishes to support.

Peanuts were sampled after 1, 8, 22, 43, 69, 97, 123, 151, 179, 207, and 235 days of daily treatment; however, only the results of analysis for days 123 and 151 days were reported. Piperonyl butoxide residues were analyzed according to the colorimetric procedures of PAM Volume II. Information pertaining to temperature, humidity, and light conditions during the peanut study was not reported. Also, the storage conditions and intervals of samples were unknown. The results are presented in Table 15.

Table 15. Residues of PBO (ppm) in/on peanut kernels following daily space treatment with PBO at a rate of 0.1 g ai/1,000 cu. ft per application for 235 days in a simulated warehouse situation.				
Number of applications (day sampled)	Row A Sample (ppm)	Row B Sample (ppm)	Row C Sample (ppm)	Average Residue (ppm)
105 (123)	<0.02	<0.02	<0.02	<0.02
133 (151)	<0.02	<0.02	<0.02	<0.02

To support postharvest uses on peanuts, data are required which depict the magnitude of PBO residues of concern in/on peanuts following applications of representative PBO formulations according to the maximum use rates the Task Force II wishes to support for: (i) space treatments of storage areas where bulk or bagged peanuts may be stored, and (ii) general space treatments of peanut processing plants.

Tobacco

The use of pesticides on tobacco does not require a tolerance or an exemption from a tolerance. Nonetheless, data are needed to assess the exposure of humans to residues on tobacco.

Data (MRID 42131903), depicting the magnitude of PBO residues in/on tobacco stored in a warehouse condition, have been submitted and reviewed (DP Barcode D179588, 12/29/92, N. Dodd). In this study, an aerosol formulation containing 0.25% esfenvalerate and 1.25% piperonyl butoxide was applied daily for 235 days at a rate of 0.1 g piperonyl butoxide per 1,000 cu. ft. According to the master label submitted by the task force, the maximum PBO use rate which the Task Force II wishes to support for general space treatments of tobacco warehouses and processing plants is 0.033 lb ai/1,000 cu. ft (14.98 g ai/1,000 cu. ft). Therefore, the PBO rate used in the tobacco study is <1.0x the rate the Task Force II wishes to support. Residues of piperonyl butoxide in/on treated boxed tobacco ranged from <0.34 ppm to <0.68 ppm on day 235. Residues of piperonyl butoxide on open tobacco increased from <0.37 ppm on day 1 to a maximum of 141.52 ppm on day 207 and were 115.42 ppm on day 235.

Since residues of piperonyl butoxide on boxed tobacco are >0.1 ppm, a tobacco pyrolysis study at a 1.0x rate must be conducted. Pyrolysis products derived from piperonyl butoxide must be characterized, and the level of the residue in smoke must be quantitated.

Summary of Magnitude of the Residue Studies – Livestock

The PBO Task Force II wishes to support direct application uses on livestock animals and their premises. In addition, the Task Force II is supporting preharvest and postharvest uses on many agricultural crops with feedstuff; thus, the potential for additional secondary transfer of PBO residues of concern in meat, milk, poultry, and eggs exists. Based upon the initial results of studies depicting the magnitude of the residue in animals as well as the observed residue levels in feedstuff from preharvest trials, the PBO uses are classified as Category 1 of 40 CFR 180.6 (a). PBO residues of concern are expected to occur in meat, milk, poultry, and eggs.

For ruminants, the Task Force II has submitted separate magnitude of the residue studies reflecting dermal (MRID 44166203) and oral (MRID 43869701) treatments. These studies have been reviewed and deemed inadequate but upgradeable; details of data gaps from these studies are listed in the respective summary of each study below. The reviewed dermal and oral studies suggest that the established tolerances for milk and ruminant tissues are too low. A premise spray treatment study with ruminants is now required for reregistration because the results of the reviewed premise study with poultry suggest that detectable residues at or near the tolerance levels are expected at 1x.

For poultry, the Task Force II has submitted separate studies reflecting oral (MRID 43869702) and premise spray (MRID 44166202) treatments. These studies have been reviewed and deemed inadequate but upgradeable. In addition, the results of a study reflecting dip treatment of PBO on laying hens are available and were summarized in a 6/29/70 memo filed in the petition file for PP#0E0977. The study is inadequate to satisfy reregistration requirements because it does not reflect the maximum use rate the PBO Task Force II wishes to support for direct application to poultry. The study was conducted using a dip solution containing 0.1% pyrethrins and 1.0% PBO whereas the Task Force II now wishes to use a 10% ready-to-use dust formulation for direct dusting of poultry. Therefore, a study reflecting direct applications on poultry using a 10% dust formulation is required for reregistration.

The Agency would have preferred that the magnitude of the residue study for animals reflect all possible exposure scenarios which include direct application to animals, premise treatment, and oral treatment. The Agency will add the residues from all possible exposure routes when the additional requested data have been submitted and reviewed; this may result in higher than necessary tolerances.

Milk and the Fat, Meat, and Meat Byproducts of Cattle, Goats, Hogs, Horses, and Sheep

Tolerances of 0.25 ppm for milk fat, reflecting negligible residues in milk, and 0.1 ppm for the fat, meat, and meat byproducts of cattle, goats, hogs, horses, and sheep have been established for residues of piperonyl butoxide [40 CFR §180.127].

Brief summaries of the available dermal and oral studies with ruminants are presented below.

Cattle dermal study (MRID 44166203)

Three Holstein dairy cows were dermally treated with an unspecified PBO formulation (ULD BP-50; EPA Reg. No. 499-453) twice daily at 2.28 ai g/day in mineral oil for 28 consecutive days. The Agency review (DP Barcode D232481, 2/1/01, T. Morton) of this study calculated the treatment rate to be equivalent to 0.8x the maximum label rate of 2.28 grams ai/animal for direct dermal application. According to Table 3, the maximum use rates which the Task Force II now wishes to support for direct application on ruminants are: 10% ai when applied with a dust formulation or as a spot-on or pour-on, 0.35 lb ai/gal when applied as a solution, and 2% ai when applied as a towelette.

Milk was collected daily, composited, homogenized, and stored frozen prior to analysis. Following the final treatment on Day 28, the cattle were sacrificed within 24 hours. Liver, kidney, composite muscle (loin and round), and composite fat (perirenal and omental) samples were collected. Tissue samples were homogenized in the presence of dry ice and then stored frozen until analysis. Samples were analyzed by a GC/MS/MS method for residues of PBO *per se*. The method was deemed adequate for data collection based on acceptable concurrent method recoveries. The results are presented in Table 16.

Table 16. Residue levels of piperonyl butoxide in milk and tissue samples of lactating dairy cattle dermally treated with piperonyl butoxide twice daily at 2.28 ai g/day for 28 consecutive days.

Collection Interval	Piperonyl Butoxide Residues (ppm)	Mean Piperonyl Butoxide Residue (ppm)
Milk		
Day-0	<0.01, <0.01, <0.01	<0.01
Day-1	0.07, 0.07, 0.03	0.06
Day-3	0.17, 0.19, 0.07	0.14
Day-7	0.14, 0.14, 0.08	0.12
Day-11	0.14, 0.11, 0.07	0.11
Day-14	0.14, 0.13, 0.07	0.11
Day-18	0.16, 0.17, 0.13	0.15
Day-21	0.18, 0.11, 0.09	0.13
Day-24	0.24, 0.17, 0.11	0.17
Day-27	0.20, 0.15, 0.13	0.16
Tissues (sacrifice)		
Muscle	0.16, 0.21, 0.16	0.18
Liver	<0.05, 0.14, <0.05	0.08
Fat	2.63, 2.70. 2.27	2.53
Kidney	0.21, 0.19, 0.21	0.20

The submitted cattle dermal study has been reviewed (DP Barcode D232481, 2/1/01, T. Morton) and deemed inadequate but upgradeable. To upgrade, the registrants are required to submit information pertaining to the storage conditions of the tissue samples and extracts as well as storage stability data reflecting the actual storage conditions of the tissue samples and extracts.

In addition, the registrants need to perform further analysis of PBO residues in milk fat since the established PBO tolerance for milk is on milk fat.

Maximum theoretical dietary burden (MTDB) - ruminants

Although certain feedstuff (i.e., field pea vines and silage) that were used in the calculations are no longer listed in Table 1 (OPPTS 860.1000), the Agency expects residues in equivalent RACs (i.e., cowpea forage and hay) to be at the same levels. The Agency will refine the estimates of dietary burdens when the requested preharvest data for many feedstuff become available. The dietary burdens are presented in Table 17.

Table 17. Calculation of maximum theoretical dietary burden (MTDB) of PBO to beef and dairy cattle.

Feedstuff	% Dry Matter [1]	Expected tolerance (ppm)	Beef Cattle		Dairy Cattle	
			% in diet	Dietary burden (ppm) [2]	% in diet	Dietary burden (ppm) [2]
Carrot culls	12	2	15	2.50	5	0.83
Field pea seed	90	1	20	0.22	20	0.22
Field pea silage	40	100	40	100.00	25	62.50
Total			100	102.72	100	63.55

[1] As per Table 1 (OPPTS 860.1000).

[2] Burden = [tolerance ÷ % DM X % diet].

Cattle oral study (MRID 43869701)

Twelve dairy cows (3 cows per dose group) were orally dosed daily with gelatin capsules containing PBO for 28-30 consecutive days at levels equivalent to 100 ppm, 300 ppm, 900 ppm, and 3,000 ppm in the diet (~1x, 3x, 9x, and 29x the maximum tentative theoretical dietary burden of PBO to ruminants, respectively).

The cattle were milked twice daily (in the a.m. and p.m.) during the acclimation and treatment period. Milk samples were collected on dose days 0 (pre-dose), 1, 3, 7, 11, 14, 18, 21, 24, and 27. For each cow, the morning and afternoon milk samples were composited and stored frozen prior to residue extraction for analysis. The treated cattle were sacrificed over a 3-day period within 16 to 24 hours after the final dose. Tissues (liver, kidney, composite round and loin muscles, and composite perirenal and omental fat) were collected and cut into small pieces and stored frozen prior to extraction for analysis. Residues in samples were analyzed by a GC/MS/MS method using a DB5 MS column. The limits of quantitation were 0.01 ppm for milk and 0.05 ppm for cattle tissues. The recovery data indicate that the GC/MS/MS method is marginally adequate for data collection because recoveries <70% were observed for egg, milk, and all tissues except fat and poultry skin. However, the average recoveries were >70% for all matrices. The results are presented in Table 18.

Table 18. Range of PBO residues found in cattle matrices following oral administration of PBO for 28-30 consecutive days at levels equivalent to 100 ppm, 300 ppm, 900 ppm, and 3,000 ppm in the diet.

Cattle Matrix	Residues of PBO (ppm)			
	100 ppm	300 ppm	900 ppm	3,000 ppm
Milk	<0.01-0.09	0.02-1.13	0.10-12.25	0.98-23.33
Liver	0.12-0.15	0.33-0.73	1.21-1.54	9.0-13.3
Kidney	<0.05	0.05-0.14	0.28-0.82	4.8-15.1
Muscle	<0.05	<0.05-0.06	<0.05-0.67	1.7-12.0
Fat	0.08-0.42	0.95-1.66	1.6-15.3	86-220

The submitted cattle oral study has been reviewed (DP Barcode D222584, 2/11/99, T. Morton) and deemed incomplete but upgradeable. To upgrade the study, the registrants are required to submit information pertaining to the storage conditions of the tissue samples and extracts as well as storage stability data reflecting the actual storage conditions of the tissue samples and extracts. In addition, the registrants need to perform further analysis on milk samples. Because the registrants believe that the variability in residue levels in milk samples is due to varying levels of milk fat in the samples analyzed, milk fat must be isolated from the samples of milk, and residues of PBO in the milk fat and the resulting skim milk must be determined.

Eggs, meat, and meat byproducts of poultry

Tolerances of 1 ppm for eggs and 3 ppm for the fat, meat, and meat byproducts of poultry have been established for residues of piperonyl butoxide [40 CFR §180.127].

Brief summaries of the available oral and premise spray studies with poultry are presented below.

Poultry oral study (MRID 43869702)

Thirty laying hens (10 hens per dose group) were orally dosed daily with gelatin capsules containing PBO at levels determined to be equivalent to 20 ppm, 60 ppm, and 200 ppm in the diet for 28 to 30 consecutive days. The MTDB of PBO to poultry was previously calculated by the Agency (DP Barcode D222584, 2/11/99, T. Morton) using the residue level of field pea seed observed from field trials reflecting preharvest treatments. Field pea seed is the only poultry feedstuff in the list of crops the Task Force II wishes to support for preharvest uses. Assuming a tolerance level of 1 ppm (based on field trial data) and a diet containing field pea seed at 20%, the maximum theoretical dietary burden of PBO to poultry was calculated at 0.20 ppm. Therefore, the feeding levels used in the submitted poultry feeding study tentatively are 100x, 300x, and 1,000x the MTDB.

Eggs were collected twice daily (in the a.m. and p.m.) during the acclimation and treatment period. Egg samples were retained on dose days 0 (pre-dose), 1, 3, 7, 11, 14, 18, 21, 24, and 27. Egg samples were composited by dose group, homogenized, and stored frozen prior to residue extraction for analysis. The treated hens were sacrificed over a 3-day period within 16 to

24 hours after the final dose; the control hens were sacrificed on day 28. The poultry tissues were cut into small pieces, homogenized using dry ice, and stored frozen prior to extraction for analysis. Samples were analyzed for residues of PBO using the previously described GC/MS/MS method with LOQs of 0.01 ppm for eggs and 0.05 ppm for tissues. The results are presented in Table 19.

Table 19. Range of PBO residues found in poultry matrices following oral administration of PBO for 28-30 consecutive days at levels equivalent to 20 ppm, 60 ppm, and 200 ppm in the diet.

Poultry Matrix	Residues of PBO (ppm)		
	20 ppm	60 ppm	200 ppm
Eggs	<0.01-0.04	0.01-0.35	<0.01-1.86
Liver	Not analyzed	<0.05	0.12-0.15
Muscle	<0.05	<0.05-0.12	0.66-0.88
Fat	0.25-0.38	0.86-1.69	10.5-13.4

The submitted poultry oral study has been reviewed (DP Barcode D222584, 2/11/99, T. Morton) and deemed incomplete but upgradeable. To upgrade the study, the registrants are required to submit information pertaining to the storage conditions of the tissue samples and extracts as well as storage stability data reflecting the actual storage conditions of the tissue samples and extracts.

Poultry premise spray study (MRID 44166202)

Ten White Leghorn laying hens were exposed to piperonyl butoxide in a premise spray. An unspecified PBO formulation (Pyrocide® Fogging Concentrate 7336; EPA Reg. 1021-1271) was applied as a premise spray for 28 consecutive days at an application rate of 5.27 g ai/1,500 cu. ft/day. The applied rate is equivalent to ~1.0x the maximum single application rate of 0.008 lb ai/1,000 cu. ft which the Task Force II wishes to support for general space treatment of poultry houses.

Eggs were collected daily, composited, homogenized, and stored frozen prior to analysis. Following the final spray treatment on Day 28, the hens were sacrificed within 24 hours. Liver, breast, thigh, skin, and fat samples were collected and composited by dose group. Tissue samples were homogenized in the presence of dry ice and then stored frozen until analysis. Samples were analyzed by a GC/MS/MS method which was deemed adequate for data collection based on acceptable concurrent recoveries. The results are presented in Table 20.

Table 20. Residue levels of piperonyl butoxide in eggs and tissue samples of laying hens following a premise spray treatment with PBO for 28 consecutive days at an application rate of 5.27 g ai/1,500 cu. ft/day.

Collection Interval	Piperonyl Butoxide Residues (ppm)	Mean Piperonyl Butoxide Residue (ppm)
Eggs		
Day-0	<0.01, <0.01, <0.01	<0.01
Day-1	<0.01, <0.01, <0.01	<0.01
Day-3	0.02, 0.01, 0.02	0.02
Day-7	0.09, 0.06, 0.03	0.06
Day-11	0.14, 0.11, 0.04	0.10
Day-14	0.21, 0.15, 0.05	0.14
Day-18	0.33, 0.14, 0.06	0.18
Day-21	0.41, 0.20, 0.11	0.24
Day-24	0.58, 0.29, 0.21	0.36
Day-27	0.79, 0.36, 0.23	0.46
Tissues (sacrifice)		
Muscle	1.20, 1.01, 0.67	0.96
Liver	0.44, 0.26, 0.15	0.28
Fat	5.04, 1.99, 1.94	2.99
Skin	8.26, 3.75, 3.30	5.10

The submitted poultry premise spray study has been reviewed (DP Barcode D232480, 2/1/01, T. Morton) and deemed inadequate but upgradeable. To upgrade the study, the registrants are required to submit information pertaining to the storage conditions of the tissue samples and extracts as well as storage stability data reflecting the actual storage conditions of the tissue samples and extracts.

Poultry dip study

The results of a study reflecting dip treatment of PBO on laying hens were summarized in a 6/29/70 memo filed in the petition file for PP#0E0977. A group of 10 hens were treated with a dip solution containing 0.1% pyrethrins and 1.0% PBO. The hens were dipped twice weekly for seven weeks. During the study period, egg samples were collected at weekly intervals, and the hens were sacrificed immediately after the 14 dip treatments. Samples were analyzed by an adequate GLC method. The results are presented in Table 21.

Table 21. Residues of PBO in eggs and tissue samples of laying hens following treatments with a dip solution containing 0.1% pyrethrins and 1.0% PBO. The hens were dipped twice weekly for seven weeks.	
Matrix/Collection Interval	Piperonyl Butoxide Residues (ppm)
Eggs	
Week 1	0.36
Week 2	0.78
Week 7	1.77
Tissues	
Skin - week 11	8.7
Body fat - week 11	2.99
Other (muscle, liver, and gizzard) - week 11	0.18 - 0.24

International Considerations

Table 22. Codex MRLs and Applicable U.S. Tolerances for Piperonyl Butoxide.			
Codex			Current U.S. Tolerance, ppm [1]
Commodity, As Defined	MRL (mg/kg)	Step	
Cattle, kidney	0.3	3	0.1 for meat byproducts of cattle, goat, hog, horse, and sheep
Cattle liver	1	3	
Cattle meat	5 (fat)	3	0.1 for meat of cattle, goat, hog, horse, and sheep
Cereal grains	30 (Postharvest or Po)	3	8 ppm for oat and sorghum resulting from postharvest uses; 20 ppm for barley, buckwheat, corn, (including popcorn), rice, rye, and wheat resulting from postharvest uses
Citrus fruits	5	3	8 ppm for oranges resulting from postharvest uses
Citrus juice	0.05	3	
Dried fruits	0.2 (Po)	3	
Eggs	1	3	1
Fruiting vegetables, cucurbits	1	3	
Kidney of cattle, goats, pigs, and sheep	0.2 (excluding cattle)	3	0.1 for meat byproducts of cattle, goat, hog, horse, and sheep
Lettuce, leaf	50	3	
Liver of cattle	1	3	0.1 for meat byproducts of cattle, goat, hog, horse, and sheep
Maize, oil, crude	80 (Po)	3	
Meat (mammalian) fat	2 (excluding cattle meat)	3	0.1 for fat of cattle, goat, hog, horse, and sheep
Milks	0.05 (excluding cattle meat)	3	0.25 ppm for milk fat
Mustard greens	50	3	
Pea hay	200	3	8 ppm for pea resulting from postharvest uses
Pea vines (green)	400	3	

Table 22. Codex MRLs and Applicable U.S. Tolerances for Piperonyl Butoxide.			
Codex			Current U.S. Tolerance, ppm [1]
Commodity, As Defined	MRL (mg/kg)	Step	
Peanut, whole	1 (Po)	3	8 ppm for peanut resulting from postharvest uses
Peppers	2	3	
Poultry meat	7 (fat)	3	3
Poultry, edible offal	10	3	3
Pulses	0.2 (Po)	3	
Radish leaves	50	3	
Root and tuber vegetables	0.5	3	0.25 ppm for potato and sweet potato resulting from postharvest uses
Spinach	50	3	
Tomato	2	3	8 ppm resulting from postharvest uses
Tomato juice	0.3	3	
Wheat	10 (Po)	3	20 ppm for barley, buckwheat, corn (including popcorn), rice, rye, and wheat resulting from postharvest uses
Wheat bran, unprocessed	80 (Po)	3	
Wheat flour	10 (Po)	3	
Wheat germ	90 (Po)	3	
Wheat wholemeal	30 (Po)	3	

[1] Very few U.S. tolerances were reassessed in the Chemistry Chapter because additional data are required for many commodities.

Table 23. Canadian MRLs for Piperonyl Butoxide.	
Commodity, As Defined	MRL (ppm)
Raw Cereals	20
Almonds, apples, beans, blackberries, blueberries, boysenberries, cherries, cocoa beans, copra, crabapples, currants, dewberries, figs, gooseberries, grapes, guavas, huckleberries, loganberries, mangoes, muskmelons, oranges, peaches/nectarines, peanuts, pears, peas, pineapple, plums, raspberries, tomatoes, and walnuts.	8
Dried codfish	1

4. TOXICOLOGY SECTION

Toxicological Endpoints

The HIARC met on April 20, 2004 to consider the toxicological endpoints and doses and other pertinent issues relating to piperonyl butoxide. A summary of the toxicological endpoints and doses selected by the HIARC (HIARC endpoint table) is presented below.

SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

Table 24. Summary of Toxicological Doses and Endpoints for Piperonyl Butoxide (PC Code 067501)

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary General Population	NOAEL= **630** mg/kg/day UF = **100** **Acute RfD** = **6.3** mg/kg/day	FQPA SF = **1X** **aPAD** = acute RfD / FQPA SF = **6.3** mg/kg/day	Developmental toxicity study, rats (Tanaka et al., 1995a)[1] LOAEL = **1065 mg/kg/day** based on decrease in maternal body weight gain
Acute Dietary Females 13-49 years	N/A	N/A	Acute Dietary Endpoint for General Population is considered protective for this population. No separate endpoint is selected.
Chronic Dietary (All populations)	NOAEL= **15.5** mg/kg/day UF = **100** **Chronic RfD** = **0.16** mg/kg/day	FQPA SF = **1X** **cPAD** = chronic RfD / FQPA SF = **0.16** mg/kg/day	Chronic oral toxicity study, dogs LOAEL = **52.8** mg/kg/day based on decrease in body weight gain, and increases in alkaline phosphatase activity, liver weight and hepatocellular hypertrophy
Short-Term Incidental Oral (1-30 days)	NOAEL= **89** mg/kg/day	**Residential** MOE = **100** **Occupational** MOE = **100**	Two generation reproduction study, rats LOAEL = **469** mg/kg/day based on the decrease in body weight gain of F_1 and F_2 pups at postnatal day 21
Intermediate-Term Incidental Oral (1-6 months)	NOAEL= **89** mg/kg/day	**Residential** MOE = **100** **Occupational** MOE = **100**	Two generation reproduction study, rats LOAEL = **469** mg/kg/day based on the decrease in body weight gain of F_1 and F_2 pups at postnatal day 21
Short-Term Dermal (1 to 30 days); Intermediate-Term Dermal (1 to 6 months); Long-Term Dermal (>6 months)	N/A Dermal Absorption = 2%	N/A	No systemic, developmental and neurotoxicity concerns at the Limit Dose. Oral NOAELs with the dermal absorption factor result in dermal equivalent doses approximately equal to or higher than the Limit Dose. Therefore, no quantification is required. PBO is classified as mild irritant. Contact should be avoided.

[1]Tanaka, T., T. Fujitani, O. Takahashi, et al., 1995a. Developmental toxicity study of piperonyl butoxide in CD rats. Toxicology and Industrial Health, 11:175-184.

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Short-Term Inhalation (1 to 30 days)	Respiratory LOAEL= **3.91** mg/kg/day (0.015 mg/L)	**Residential** MOE = **300** **Occupational** MOE = **300**	Subchronic inhalation toxicity study, rats Respiratory LOAEL = **3.91** mg/kg/day (0.015 mg/L) based on laryngeal hyperplasia and metaplasia
Intermediate-Term Inhalation (1 to 6 months)	Respiratory LOAEL= **3.91** mg/kg/day (0.015 mg/L)	**Residential** MOE = **300** **Occupational** MOE = **300**	Subchronic inhalation toxicity study, rats Respiratory LOAEL = **3.91** mg/kg/day (0.015 mg/L) based on laryngeal hyperplasia and metaplasia
Long-Term Inhalation (>6 months)	Respiratory LOAEL= **3.91** mg/kg/day (0.015 mg/L)	**Residential** MOE = **1000** **Occupational** MOE = **1000**	Subchronic inhalation toxicity study, rats Respiratory LOAEL = **3.91** mg/kg/day (0.015 mg/L) based on laryngeal hyperplasia and metaplasia
Cancer	NA	NA	Classified as "Group C carcinogen" with RfD approach for quantification

NOTE: The Special FQPA Safety Factor recommended by the HIARC **assumes** that the exposure databases (dietary food, drinking water, and residential) are complete and that the risk assessment for each potential exposure scenario includes all metabolites and/or degradates of concern and does not underestimate the potential risk for infants and children.

UF = uncertainty factor, FQPA SF = Special FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure, NA = Not Applicable

NOTE: The Special FQPA Safety Factor recommended by the HIARC **assumes** that the exposure databases (dietary food, drinking water, and residential) are complete and that the risk assessment for each potential exposure scenario includes all metabolites and/or degradates of concern and does not underestimate the potential risk for infants and children.

1. Acute Reference Dose (aRfD) - General population

Proposed Study: §

Rats - Developmental Study

MRID No.: None

Executive Summary:

In a developmental toxicity study reported by Tanaka et al. (1995), piperonyl butoxide was administered to Crj:CD rats (10 females/dose in all groups except 15 females in controls) by

gavage at dose levels of 0, 630, 1065, or 1800 mg/kg/day on days 11-12 of gestation. Maternal body weight gain was significantly less ($p<0.01$) in mid and high dose groups as compared to controls. The maternal body weight gain was 24% and 37% of controls in mid and high dose groups, respectively. Total resorption rate increased significantly in high dose group ($p<0.001$) compared to controls. The resorption rate was reported as 0.56, 0.00, 2.10, and 35.3% in control, low, mid and high dose groups. The number of viable fetuses decreased in high dose group as compared to controls (99 in high dose versus178 in controls). The number of fetuses with limb deformities was 27 and 44 in mid and high dose groups as compared to none in control and low dose groups. The limb deformities included oligodactyly, syndactyly, polydactyly and combinations of those. **The maternal NOAEL/LOAEL is 630/1065 mg/kg/day based on decreased maternal body weight gain. The developmental NOAEL/LOAEL is determined as 630/1065 mg/kg/day based on limb deformities in the fetuses. At the high dose tested increased resorption rate and decreased number of viable fetuses were noticed.**

Proposed Dose and Endpoint for Establishing aRfD:

The proposed dose of 630 mg/kg/day (maternal NOAEL) is based on the decrease in body weight gain during gestation at the LOAEL of 1065 mg/kg/day.

Proposed Uncertainty Factor (UF): 100 (10x for interspecies and 10x for the intraspecies)

Comments about Study/Endpoint/Uncertainty Factor:

Although the study reported the body weight changes after two gavage doses during gestation days 11-12, it is reasonable to assume that effects could have occurred after a single dose administration and thus considered appropriate for Acute Reference Dose selection. Significant decrease in body weight gain during gestation days 6-9 was reported in the Guideline study (MRID 42380801), i.e., body weight gain decreased to 22.8%, 13.1 % and 50.8% in 200, 500 and 1000 mg/kg/day groups, as compared to controls. However, the guideline study was not selected because the effects were not dose dependent. Also, there was no significant body weight effects in the subsequent gestation intervals (9-12 days, 12-15 days, 6-15 days) at the low dose and the effects in mid dose during gestation days 6-9 was equivocal. The high dose effects with regard to body weight gain in the Guideline study are considered significant, however, this dose is close to the LOAEL (1065 mg/kg/day) established in the study proposed for endpoint selection.

Acute RfD (General population) = $\frac{630 \text{ mg/kg/day (NOAEL)}}{100 \quad \text{(UF)}}$ = **6.3 mg/kg/day**

2. Chronic Reference Dose (cRfD)

Proposed Study: §

Chronic Oral Toxicity - Dog

MRID No. 42926001, 42926002
Executive Summary:

In a chronic toxicity study (42926001, 42926002) piperonyl butoxide (90.78%, Lot number FEP-100) was administered to beagle dogs (4/sex/dose) at 0, 100, 600 or 2000 ppm for one year. The corresponding chemical intake values for PBO treated groups were 2.9, 15.5, or 52.8 mg/kg/day in males and 2.7, 16.3 or 71.0 mg/kg/day in females. A significant decrease in body weight gain was noticed in high dose animals. Males had a body weight gain of 24.8 %, 21.2%, 16%, and 3.1% of the initial weights in control, low, mid and high dose groups. Females had a body weight gain of 21.8%, 24.8%, 19.8% and -2.0% (lost) in the control, low, mid and high dose groups. Decrease in food consumption paralleled the body weight changes in males but not in females.

At 2000 ppm, serum alkaline phosphatase was elevated in males (340% at 6 months and 539% at 1 year) and females (368% at 6 months and 512% at 1 year). Mid dose males had a 60% elevation in alkaline phosphatase activity at 6 months and during termination, as compared to controls. In contrast, the mid dose females had a 23% and 10% decrease in alkaline phosphatase activity at 6 months and one year, relative to the respective controls. Since the increase in alkaline phosphatase activity occurred only in mid dose males, the enzyme changes in mid dose group are not considered significant.

Terminal high dose group liver weight was also increased in males (22% absolute and 52% relative) and females (36% absolute and 86% relative). Mid dose females had a slight increase in liver weight (12% absolute and 19% relative) as compared to controls. Increase in liver weight in mid dose males was not significant as compared to controls (3% absolute and 13% relative) Females in 2000 ppm had a 35% increase in absolute thyroid/parathyroid weight and 83% increase in thyroid/parathyroid weight relative to body weight. Hepatocellular hypertrophy was reported in 75% of the high dose males (3/4) and 100% of females.

The LOAEL is 2000 ppm (52.8 mg/kg/day) based on decreases in body weight gain, increased alkaline phosphatase activity and increased liver weight and hepatocellular hypertrophy. The NOAEL is established as 600 ppm (15.5 mg/kg/day).

The chronic feeding study in dogs is **Acceptable/Guideline** and satisfies the guideline requirement for a chronic oral study (OPPTS 870. 4100, OECD 452) in dogs.

Proposed Dose and Endpoint for Establishing cRfD:

The dose of 15.5 mg/kg/day based on decreases in body weight gain, increased alkaline phosphatase activity, increased liver weight and hepatocellular hypertrophy at the LOAEL of

52.8 mg/kg/day.

Proposed Uncertainty Factor(s): 100 (10x interspecies factor and 10x intraspecies factor)

Comments about Study/Endpoint/Uncertainty Factor:

This study is selected because 1) dogs appear to be the more sensitive species for the toxic effects of piperonyl butoxide than rats and mice 2) it is appropriate study for the duration and route of exposure 3) the study has endpoints similar to that reported in other chronic studies such as combined chronic and carcinogenicity studies in mice and rats for decreased body weight gain, liver weight and liver histopathological effects and 4) there are no developmental and reproductive concerns at the doses selected for risk assessment.

Chronic RfD = $\frac{\text{15.5 mg/kg/day (NOAEL)}}{\text{100 (UF)}}$ = **0.155 mg/kg/day**

3. Classification of Carcinogenic Potential

Carcinogenicity Peer Review Committee (CPRC) evaluated the tumor data on piperonyl butoxide in February 1995 and concluded that piperonyl butoxide should be classified as a Group C -possible human carcinogen based on statistically significant increases in hepatocellular tumors in both sexes of CD-1 mouse (adenomas, carcinomas, and combined adenomas/carcinomas in males and adenomas in females) (TX011576). Piperonyl butoxide induced liver tumors (hepatocellular adenoma and carcinoma) in rats at excessive doses (>1000 mg/kg/day).

4. Mutagenicity

Piperonyl butoxide has been tested in several mutagenicity studies. The bacterial gene mutation assays indicate no induction of mutation in cells treated with piperonyl butoxide. The *in vitro* mammalian cell mutation assays conducted using CHO cells indicate a questionable positive effect in the absence of metabolic activation. However, a negative response to induction of mutation was observed in the presence of metabolic activation in CHO cells. NTP testing for piperonyl butoxide in mouse lymphomas cells indicate positive effect for mutation. But the activation conditions for positive mutation effects in lymphomas cells are not presented. Piperonyl butoxide tested negative for chromosomal aberration and sister chromatid exchange in CHO cells. In rat primary hepatocytes, no induction of unscheduled DNA synthesis was observed by piperonyl butoxide treatment close to cytotoxic concentrations.

5. Neurotoxicity

EPA guideline neurotoxicity studies are not available for PBO. Neurotoxic effects of PBO are not evident from the clinical signs reported in developmental, reproductive and chronic studies submitted to the Agency. The clinical signs observed in rat developmental range finding study (MRID 42586901) included urogenital area wetness and perinasal encrustation at 500 and 1000 mg/kg/day and these effects were not reported consistently in other toxicity studies.

The possible examples of neurotoxicity due to PBO are shown below.

In the reproduction toxicity studies (Tanaka,1992, and Tanaka et al., 1992) conducted in mice, impairment in certain neurobehavioral functions (sensory and locomotor function) were reported. Decreased ambulation and rearing in parents and decreased olfactory orientation in pups were observed upon exposure to piperonyl butoxide. The results are summarized below.

In a single generation reproduction study (Tanaka, 1992), piperonyl butoxide was administered to Crj:CD1 mice (10/sex/dose) in the diet at 0, 1500, 3000, or 6000 ppm from 5 weeks of age in the F_0 generation to 9 weeks of age in the F_1 generation. The open field test results indicate a dose dependent decrease in ambulation in F_0 generation male mice at 8 weeks of age (i.e., after 3 weeks of exposure) and the results were significant ($p<0.01$) at 6000 ppm. Rearing was also affected in a dose dependent manner in F_0 generation male mice at 8 weeks of age, however, the decrease in rearing was not statistically significant. A sporadic decrease in ambulation and rearing was observed in piperonyl butoxide treated F_1 males and females at 3 weeks of age and these functions were not affected at 8 weeks of age. A significant decrease in olfactory orientation ($p<0.01$) in F_1 mice was observed at 2 weeks in 3000 and 6000 ppm groups. **The parental NOAEL/LOAEL is determined as 3000/6000 ppm based on decreased ambulation and possibly, decreased rearing in F_0 male mice. The offspring NOAEL/LOAEL is determined as 1500/3000 ppm based on decrease in olfactory orientation in F_1 mice.**

6. Developmental and Reproductive Toxicity

In a developmental study (MRID 42380801) piperonyl butoxide (90.78% a.i., Lot No: FEP-100) was administered to 25 female Crl:CD rats/dose by gavage in deionized water at dose levels of 0, 200, 500 and 1,000 mg/kg/day from days 6 through 15 of gestation.

During gestation days 6-9, body weight gain decreased to 22.8%, 13.1 % and 50.8% and food consumption decreased to 9.7%, 14.9 and 21.0% in 200, 500 and 1000 mg/kg/day groups, respectively, as compared to controls. Significant ($p<0.05$) decrease in body weight gain (18.3-21.8%) and food consumption (7.8-9.1%) were noticed during gestation days 6-15 in 500 and 1000 mg/kg/day groups. Decrease in body weight gain and food consumption was 9% and 5% in low dose group as compared to controls and the results are not statistically significant. Increased incidence of urogenital wetness (54%), urine stains (12.5%), red discharge (4%) and perinasal encrustation (8%) were observed in 1000 mg/kg/day group. **The maternal LOAEL is 500**

mg/kg/day based on decrease in body weight gain and food consumption during gestation days 6-15. The maternal NOAEL is 200 mg/kg/day.

A dose dependent increase in the incidences of unossified cervical centrum #5 and cervical centrum#6 was reported in fetuses of piperonyl butoxide treated animals. The percent incidences of unossified cervical centrum #5 in pups/litter were 9.4/33.3, 20.8/61.9, 29.2/82.6 and 36.2/79.2 in control, low, mid and high dose groups, respectively. Similarly, the percent incidences of unossified cervical centrum #6 in pups/litter were 9.9/41.7, 15.4/52.4, 21.7/56.5 and 31.6/75.0 in control, low, mid and high dose groups. These effects were considered not biologically significant in the original review (TXR 0010108). A further evaluation indicates that the percent incidences of unossified cervical centrum #5 and #6 in mid and high dose groups could be related to treatment due to dose dependent effects and the incidences exceeding that of the concurrent controls. Although the historical controls have a wide range of incidences for the unossified cervical centrum #5 and #6, the effects in mid and high dose groups are above the mean incidences for historical controls for unossified cervical centrum #5 (22.6% in fetus and 61.3% in litter) and unossified cervical centrum #6 (19.1% in fetus and 55.8% in litter) and are considered treatment related. **Therefore, the developmental LOAEL is 500 mg/kg/day based on increased incidences of unossified cervical centrum #5 and #6 in fetuses and litter. The developmental NOAEL is 200 mg/kg/day**.

In a range finding study (MRID 42586901), 100% mortality of pregnant dams were reported in 2000 and 4000 mg/kg/day groups. All 5 dams in the 4000 mg/kg/day group and 4 dams in 2000 mg/kg/day group were sacrificed moribund between gestation days 7 and 8. Decreased body weight gain and clinical signs were noticed in 500 and 1000 mg/kg/day groups. The clinical signs included urogenital area wetness and perinasal encrustation.

The developmental toxicity study in rats is **Acceptable/Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700a; OECD 414) in the rat.

Rabbit

In a developmental toxicity study (MRID 00157157) piperonyl butoxide (100% a.i., Lot No:LS85-58) was administered to 16 female New Zealand White rabbits/dose by gavage at dose levels of 0, 50, 100 and 200 mg/kg/day from days 7 through 19 of gestation.

During gestation days 7-19, the respective mean body weight gain in control, low, mid and high dose animals were 39(±200), 111 (±102), -19 (±160) and -159 (±204) grams with standard deviation given in parentheses. Although the high dose animals appear to have lost significant body weight gain when compared to controls, the body weight loss in high dose group is not considered significant due to huge variations. Also, the mean body weight gains in piperonyl butoxide treated rabbits are not different from controls during gestation days 0-29. The mean body weight during the whole gestation period (0-29) was 200 (±202), 300 (±197), 273 (±149) and 217 (±154) grams for control, low, mid and high dose animals, respectively. The symptoms of defecation were noticed in mid and high dose groups (37.5% each; 6/16 animals) while low dose and controls had 0% (0/16) and12.5% defecation (2/16 rabbits), respectively. The defecation symptoms appear to be incidental and not related to treatment. **The maternal**

NOAEL is 200 mg/kg/day, the highest dose tested. The maternal LOAEL is >200 mg/kg/day.

Skeletal examinations revealed the presence of 27 presacral vertebrae. The numbers of fetuses/litter for 27 presacral vertebrae were: 25/9, 41/12, 29/9 and 54/14 for control, low, mid and high dose groups, respectively. The corresponding percentage of fetuses and litters affected for 27 presacral vertebrae were: 20/56, 32/75, 27/64 and 62/88 for control, low, mid and high dose groups. No other treatment related developmental effects were observed up to 200 mg/kg/day treatment. The presence of 27 presacral vertebrae was not dose dependent and considered not biologically significant. **The developmental NOAEL is 200 mg/kg/day, the highest dose tested. The developmental LOAEL is >200 mg/kg/day.**

In a 2-generation reproduction study (MRID 00161118) piperonyl butoxide (88% a.i., Lot # FEG-32) was administered to 26 CD-Crl-COBS CD [SD] BR rats/sex/dose in diet, at dose levels of 0, 300, 1000, or 5000 ppm. The corresponding average chemical intake values prior to gestation for both generations were 27/30, 89/102, or 469/528 mg/kg bw/day in males/females, respectively. The reproductive toxic effects of piperonyl butoxide were measured in two litters for each generation (F1a and F1b; F2a and F2b).

Body weight gain was consistently lower in the high dose parental groups and it corresponded to 6-12% for F_0 males, 9% for F_0 females, 12% for F_1 males and 9% for F_2 females. **The parental systemic LOAEL is 5000 ppm (469 mg/kg/day in males, 528 mg/kg/day in females) based on decreased body weight gain. The parental systemic NOAEL is 1000 ppm (89 mg/kg/day in males, 102 mg/kg/day in females).**

Although the weights of the pups in high dose group were comparable to controls at birth, a small but consistent decrease in body weights was noticed at post-natal day 4 and the effects were pronounced at post-natal day 21. At post-natal day 4, the percent decrease in pup weight in the high dose group was 8 % in F_1a pups, 5-9% in F_1b pups, 6-13% in F_2a pups and 12% in F_2b pups as compared to controls. At post-natal day 21, the percent decrease in pup weights relative to controls corresponded to 18 % in F_1a pups ($p<0.001$), 12-15% in F_1b pups ($p<0.001$), 10-13% in F_2a pups (not significant in males but significant at $p<0.01$ in females) and 18-19% in F_2b pups ($p<0.001$) as compared to controls. **The offspring systemic LOAEL is 5000 ppm (469 mg/kg/day in males and 528 mg/kg/day in females) based on decreased body weight gain in F_1 and F_2 pups at post-natal day 21. The offspring NOAEL is 1000 ppm (89 mg/kg/day in males and 102 mg/kg/day in females).**

There were no treatment-related effects in reproductive measurements (mating index, fertility index, gestation length etc.). **The reproductive NOAEL is 5000 ppm (469 mg/kg/day in males, ≥528 mg/kg/day in females), the highest dose tested.**

Additional Information from Literature Sources

Developmental Toxicity

Rats

Study 1

In a developmental study reported by Kennedy et al. (1977), piperonyl butoxide was administered to COBS random bred albino rats at 0, 300 or 1000 mg/kg/day (20 females/dose) by gavage during 6 to 15 days of gestation. The pregnant dams in the piperonyl butoxide treated groups had a reduction in mean body weight (10-15% of controls) during gestation days 6-20. No developmental toxic effects were observed at the doses tested. **The maternal NOAEL/LOAEL is 300/1000 mg/kg/day based on reduction in mean body weight during gestation. The developmental NOAEL is determined as 1000 mg/kg/day, the highest dose tested.**

Study 2

In a developmental toxicity study reported by Tanaka et al. (1995), piperonyl butoxide was administered to Crj:CD rats (10 females/dose in all groups except 15 females in controls) by gavage at dose levels of 0, 630, 1065, or 1800 mg/kg/day on days 11-12 of gestation. Maternal body weight gain was significantly less ($p<0.01$) in mid and high dose groups as compared to controls. The maternal body weight gain was 24% and 37% of controls in mid and high dose groups, respectively. Total resorption rate increased significantly in high dose group ($p<0.001$) compared to controls. The resorption rate was reported as 0.56, 0.00, 2.10, and 35.3% in control, low, mid and high dose groups. The number of viable fetuses decreased in high dose group as compared to controls (99 in high dose versus178 in controls). The number of fetuses with limb deformities was 27 and 44 in mid and high dose groups as compared to none in control and low dose groups. The limb deformities included oligodactyly, syndactyly, polydactyly and combinations of those. **The maternal NOAEL/LOAEL is 630/1065 mg/kg/day based on decreased maternal body weight gain. The developmental NOAEL/LOAEL is determined as 630/1065 mg/kg/day based on limb deformities in the fetuses. At the high dose tested increased resorption rate and decreased number of viable fetuses were noticed.**

Mice

Study 1

In a developmental toxicity study reported by Tanaka et al. (1994) piperonyl butoxide was administered to Crj:CD-1 mice (20 females/dose) by gavage at dose levels of 0, 1065, 1385, and 1800 mg/kg/day as only a single dose on day 9 of gestation. No change in maternal body weight gain was reported. Early and late fetal deaths were significantly increased in the mid and high dose groups ($p<0.001$). The mean total resorption rate was 5.5, 7.46, 26.2 and 32.6 % in control, low, mid and high dose groups, respectively. The percent incidence of oligodactyly of forelimbs was 0, 0.4, 2.0 and 15.6 in control, low, mid and high dose groups. **The maternal NOAEL is greater than 1800 mg/kg/day. The developmental NOAEL/LOAEL for this study is**

1065/1385 mg/kg/day based on early and late fetal deaths and increased resorption rate in mice. At the high dose tested, (1800 mg/kg/day) increased incidences of limb deformities in fetuses were present.

Reproduction Effects

Mice

Study 1

In a single generation reproduction study (Tanaka, 1992), piperonyl butoxide was administered to Crj:CD1 mice (10/sex/dose) in the diet at 0, 1500, 3000, or 6000 ppm. Significant ($p<0.001$) reductions in body weight of the pups were observed at birth and at 4, 7, 14 and 21 postnatal days in high dose. Significant ($p<0.001$) reductions in body weight of the pups were also noticed at postnatal days 7 and 14 in mid dose. **The offspring NOAEL/LOAEL was determined as 1500/3000 ppm based on decreased pup weight at post-natal day 7 and 14. The parental NOAEL/LOAEL is determined as 3000/6000 ppm based on decreased ambulation and possibly, decreased rearing in F_0 male mice.**

Study 2

In a two-generation reproduction study, (Tanaka et al., 1992) piperonyl butoxide was administered to Crj:CD1 mice (10/sex/dose) in the diet at 0, 1000, 2000, 4000 or 8000 ppm. The respective chemical intake concentrations during mating period were reported as 159, 317, 648, and 1237 mg/kg/day for F_0 generations. The corresponding chemical intake concentrations for F_1 generation were: 171, 319, 665, and 1341 mg/kg/day.

The parental toxic effects except for food consumption data were not reported. The food consumption in the F_0 parents and F_1 parents during mating period and gestation were not affected by piperonyl butoxide treatment. Decreased food consumption was noticed in F_1 generation and F_2 generation mice during lactation at 8000 ppm. Decrease in number of litters, number of pups, litter size and litter weight were reported at 8000 ppm for both F_1 and F_2 generations. Decreases in litter size and weight were also reported at the lower dose level (4000 ppm) in F_2 generation. Pup weights during lactation phase were significantly reduced in both F_1 and F_2 generations at 4000 and 8000 ppm groups. The percentage of surviving pups at 21 days for either sex was reduced to 63% in F_1 generation and 59% in F_2 generation in 8000 ppm groups as compared to 91-100% in the respective controls.

The offspring NOAEL/LOAEL is determined as 2000/4000 ppm (319/665 mg/kg/day) based on reduction in litter size and litter weight in F_2 generation and decreased F_1 and F_2 pup weight during lactation. Decreased food consumption during lactation, number of litters, litter size and litter weight, number of pups at birth and pup weight and survival of pups during lactation were reported at 8000 ppm for both F_1 and F_2 generations.

Rat Metabolism

In a metabolism study (MRID 45582701), a mixture of non-radiolabeled (93.4 % a.i.; Lot/Batch No. SF-97-004) and phenyl labeled ^{14}C-piperonyl butoxide (100% radiochemical purity; Lot/Batch No. 980319/RP2) was administered to 4 CRL:CD rats/sex/dose by single gavage exposure at dose levels of 50 or 500 mg/kg body weight. The main route of excretion was via feces which contained 82.9-85.1% of the administered radioactivity at the low dose level and 64.1-75.9% at the high dose level at 168 hours. The percent radioactive dose excreted in the urine during 168 hours was 11.1-14.4% in low dose group and 19.5-30.2% in the high dose group. The majority of the administered radioactivity was excreted in 0-48 hour urine and feces samples in both dose groups. The percent of administered dose in carcass was below 0.5% in either low dose or high dose groups. The total percent of radioactive dose recovered in both dose groups ranged between 97.4% and 99.6%. There is no significant difference in the excretion pattern either between two dose groups or between sexes in the same dose group.

M1 and M3 are the major metabolites excreted in feces. M1 was identified as unchanged PBO corresponding to 15.6-23.9% of administered dose. M3 was identified as PBO with methylenedioxy ring opened to form catechol and found at 17.4-19.7% of the administered dose. M2 and M4/M5 were also identified but were present in low amounts (4-6% of administered radioactivity) in high dose group.

Several radioactive peaks (~20 peaks) were observed in urine samples and none of these individual peaks exceeded 5% of the administered radioactivity. The significant metabolite in male urine was found to be M14 which occurred at 3% of the administered dose. In females, the significant urinary metabolites identified were M6 and the combined M7/M8 which contained 5 and 9% of the administered dose, respectively. Although there was no significant difference in the excretion of metabolites between two dose groups, metabolites M5, M8, M9 and M10 were predominantly found in female urine samples and M14 was reported only in male urine samples.

Based on the identification of metabolites, the authors proposed three major reactions in the metabolism of PBO: 1) Opening of the methylenedioxy ring to form the catechol; 2) Sequential cleavage of the 2-(2-butoxyethoxy)ethoxymethyl side chain to produce series of alcohols and acids; 3) Conjugation of one of the phenolic groups to yield glucuronide, sulphate or methoxy derivative.

This study provides additional clarification on the characterization of metabolites reported earlier (MRID 41998701 and 41998401). Together with the previously submitted information, the metabolism study in the rats is classified as acceptable guideline, and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats. The metabolic profile in presented in Table 25.

TABLE 25. Metabolite profile in excreta of rats dosed with C^{14}-labeled Piperonyl Butoxide[a].

Dose	Percent of administered dose (500 mg PBO/Kg Body weight)					
	Urine		Feces		Urine and Feces	
Compound	Male	Female	Male	Female	Male	Female
Parent or M1	-	-	23.9	15.57	23.9	15.57
Metabolite M2	-	-	3.74	4.36	3.74	4.36
Metabolite M3	0.11	0.21	19.69	17.36	19.8	17.57
Metabolite M4	-	-	4.68	6.28	4.68	6.62
Metabolite M5	-	0.34		-		
Metabolite M6	1.32	4.98	-	-	1.32	4.98
Metabolite M7		9.27	-	-		9.27
Metabolite M8	-		-	-	-	
Metabolite M9	-	0.62	-	-	-	0.62
Metabolite M10	-	0.28	-	-	-	0.28
Metabolite M11#	-	-	-	-	-	-
Metabolite M12#	-	-	-	-	-	-
Metabolite M13*	0.08	0.33	-	-	0.08	0.33
Metabolite M14	3.07	0.78	-	-	3.07	0.78
Metabolite M15*	1.82	1.34	-	-	1.82	1.34
Metabolite M16	0.78	0.98	-	-	0.78	0.98
Metabolite M17#	-	-	-	-	-	-
Total	7.18	19.1	52	43.6	59.2	62.7

[a] Data obtained from page 38 of the study report.

* Metabolites that were detected by mass spectrometry but structures could not be determined from the mass data.

Metabolites were identified by mass spectrometry but were not present in sufficient concentrations to be detectable or quantifiable by HPLC

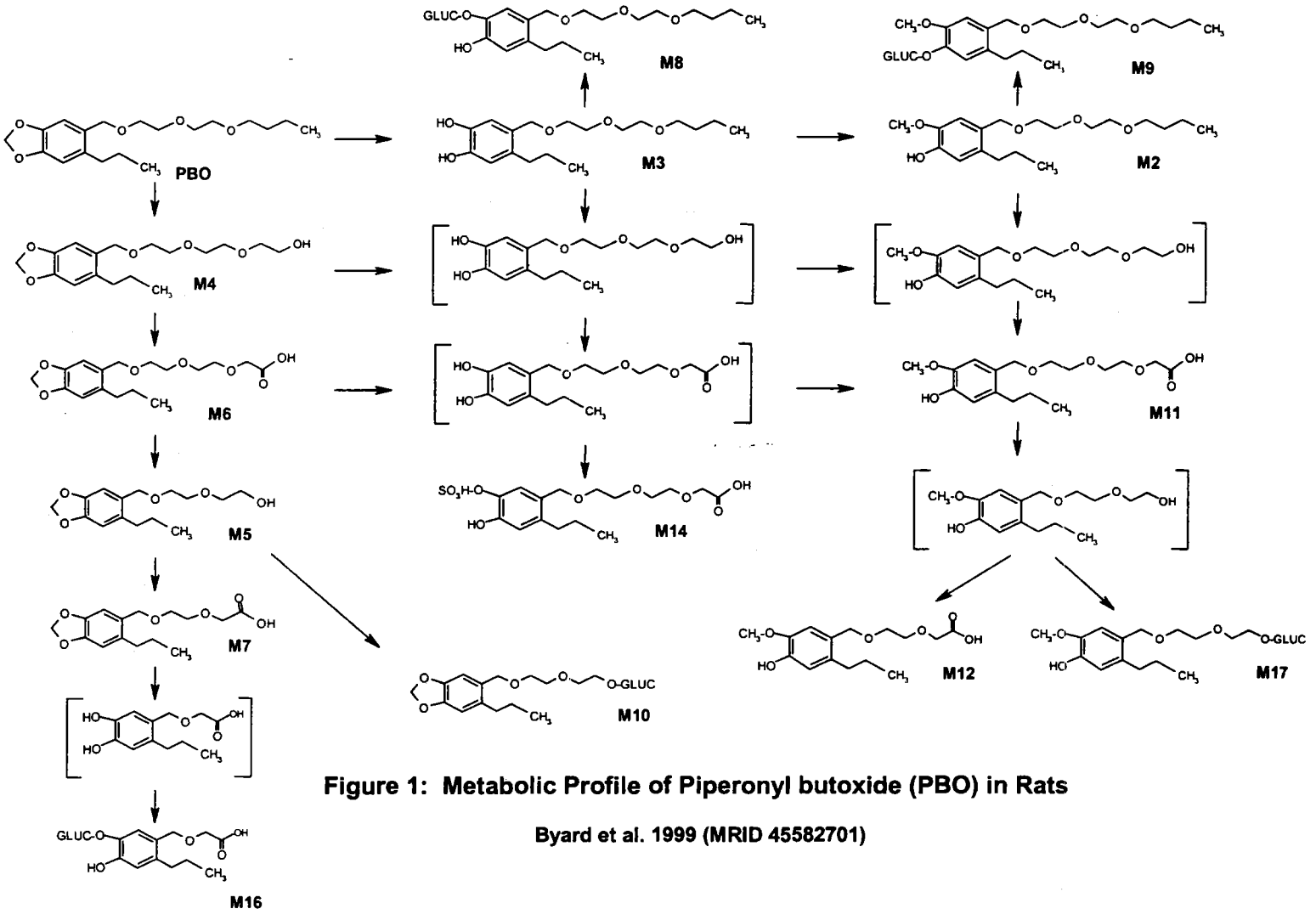


Figure 1: Metabolic Profile of Piperonyl butoxide (PBO) in Rats

Byard et al. 1999 (MRID 45582701)

Note: The structures in brackets indicate potential intermediates which were not identified. The methyl, glucuronide (GLUC) and sulfate groups on the phenyl ring could be conjugated to either of the hydroxyl groups and only one is shown in the figure.

5. RESIDUES IN WATER

Environmental Persistence

Piperonyl butoxide ("PBO," $C_{19}H_{30}O_5$, molecular weight 338.45) is a liquid at room temperature, and boils at 180°C (1 mmHg). Its solubility in water is 14.34 µg/mL, and the log octanol-water partition coefficient is 4.95. Its estimated vapor pressure is 1×10^{-7} mmHg at 25 °C, and its estimated Henry's Law constant is 8.89×10^{-11} atm-m^3/mol at 25 °C. The estimated second-order reaction rate constant with atmospheric hydroxyl radicals is 1.07×10^{-10} cm^3/molecule-sec. (SRC PhysProp database).

Piperonyl butoxide degrades in the environment by photolysis in water (half-life 8.4 hours), and is metabolized by soil microorganisms (half-life 14 days). Other tested routes of degradation are very slow (hydrolysis, aerobic and anaerobic aqueous metabolism) or have questionable rates due to experimental difficulties (soil photodegradation). The estimated atmospheric half-life of PBO is 3.4 hours, based on the estimated reaction rate with hydroxyl radicals.

Expected Mobility

PBO is moderately mobile in soil-water systems, with Koc values of 399 to 830, and a solubility of 14 ppm.. Little volatilization from soil or water is expected based on the low Henry's Law constant, however PBO may enter the atmosphere as an aerosol as a result of spraying. Based on its log Kow (4.75) moderate bioconcentration (estimated BCF 1,100) in aquatic organisms is expected (WM Meylan and PH Howard, J Pharm Sci 84: 83-92, 1995).

The studies of unaged column leaching in sand, clay loam, sandy loam, and silt loam soils were scientifically valid, but K_d values could not be calculated due to limited movement of the parent compound. PBO (parent) was mobile only in the sand soil (K_d = 0.42 mL/g).

Environmental Degradates

The major degradates PBO-alcohol, PBO-aldehyde, and PBO-acid are expected to be more soluble in water and therefore more mobile in soil-water systems than the parent, based on their lower molecular weights and hydrophilic moieties. The reported solubility of piperonal, which differs from PBO-aldehyde by lacking the propyl side chain, is 1 part in 500 parts water (Merck Index 10th Ed., 1983, monograph 7350), or 2000 parts per million. Piperonylic acid (monograph 7352) which differs from PBO-acid by lacking the propyl side chain is "slightly" soluble in water.

Minor degradates include PBO prop-1-one (M16); PBO prop-1-one benzaldehyde (M11); 5[2-(2-butoxyethoxy)-hydroxymethyl]-6-carboxy-1,3-benzodioxole (M8); and the PBO-alcohol dimer. All of these are pictured below in the degradation schemes (Figure 2).

The maximum levels of degradates found in the environmental fate studies for piperonyl butoxide (PBO are given in Table 26. These data are taken from the Photodegradation in Water (MRID 43637201), Photodegradation on Soil (43720801), Aerobic Soil Metabolism (43806401), Aerobic Aquatic Metabolism (43803401), and Anaerobic Aquatic Metabolism (43836501) studies.

Structures and names of the degradates identified in these studies are also provided.

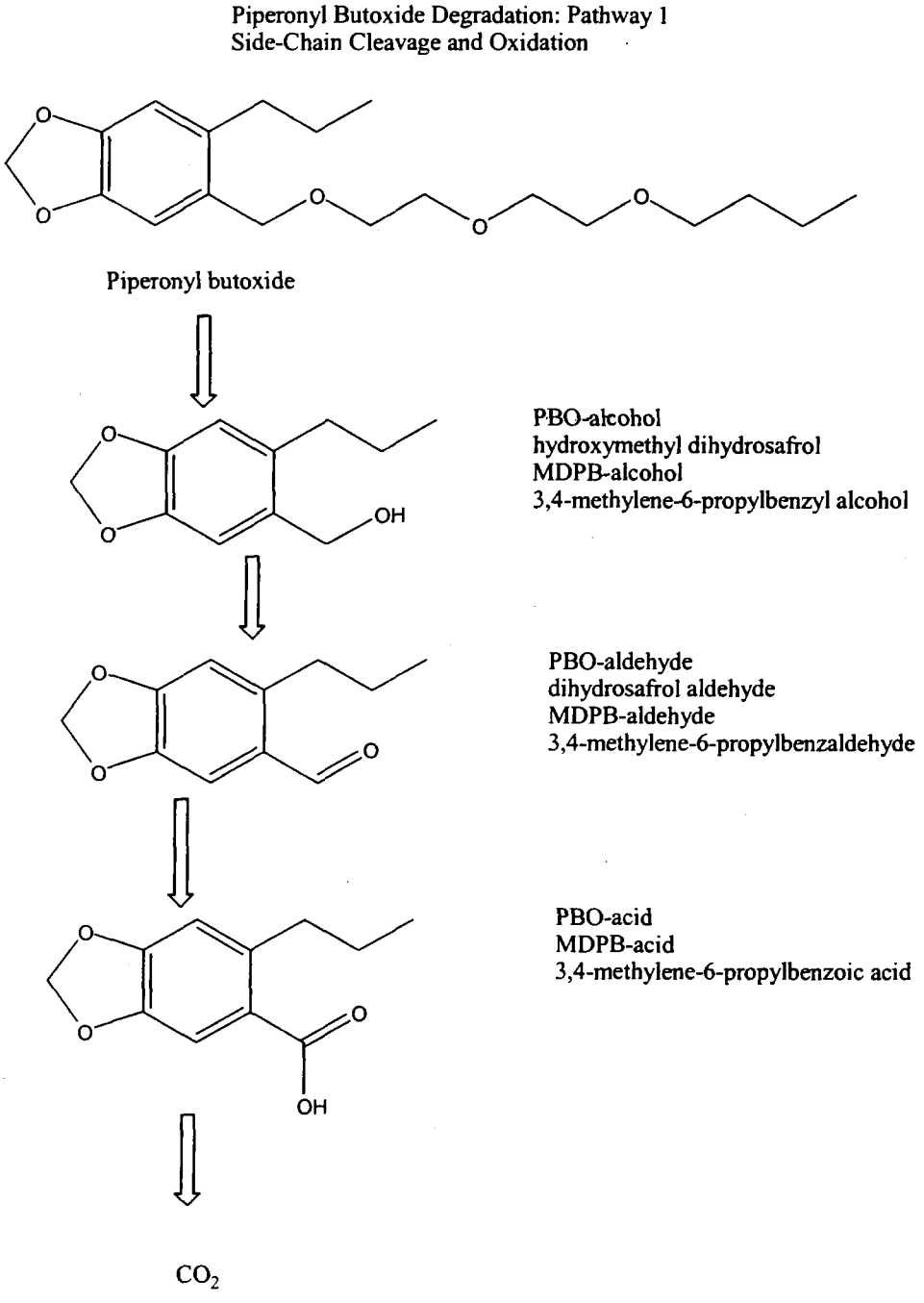
The first side-chain degradate (Butyl Carbitol) is the other product formed when PBO is cleaved to form PBO-alcohol (p. 3 of attachment). This compound is "used as a marker for the degree of photodegradation...of PBO." (C.A.J. Harbach et al., *Photolytic Degradation of Piperonyl Butoxide*, chapt. 6 in Piperonyl Butoxide The Insecticide Synergist, D.G. Jones, ed. Academic Press, 1998). Its initial concentration should therefore be the same as PBO-alcohol. The other two side-chain degradates are also formed by ether cleavage.

Table 26. Maximum Concentrations of PBO Degradates

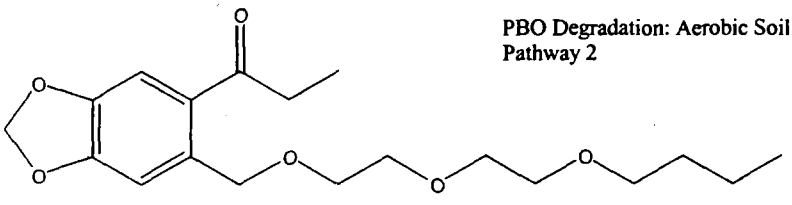
Degradate Name (a)	Photodeg/ Water	Photodeg/ Soil (b)	Aerobic Soil	Aerobic Aquatic	Anaerobic Aquatic
Application Rate	8.6 ppm	100 μg/cm^2 ≡10 kg/ha	10 ppm	10 ppm	10.2 ppm
Half-life	8.4 hours	not calculated (b)	14 days	213 days	stable
PBO-alcohol	**4.7 ppm (54.7%) @ 36 hr**	**4.41 kg/ha (44%) @ 3 days**	--	(water) 0.38 ppm (3.8%) @ 30 days (sed) 0.075 ppm (0.8%) @ 30 days	(w) ≤0.04 ppm (s) ≤0.10 ppm
PBO-aldehyde	**1.0 ppm (11.6%) @ 36 hr**	0.76 kg/ha (7.6%) @ 6 days	--	(w) 0.18 ppm (1.8%) @ 30 days (s) 0.09 ppm (0.9%) @ 21-30 days	(w) ≤0.12 ppm (s) --
PBO-acid	--	**0.98 kg/ha (9.8%) @ 10 days**	**1.7 ppm (17%) @ 30 days**	(w) 0.34 ppm (3.4%) @ 30 days (s) 0.15 ppm (1.5%) @ 30 days	(w) ≤0.15 ppm (s) ≤0.10 ppm
PBO-alcohol dimer	–	0.48 kg/ha (4.8%) @3 days	–	–	--
PBO prop-1-one	--		0.3 ppm (3%) @ 30 days	--	--
PBO prop-1-one benzaldehyde	--		0.58 ppm (5.8%) @ 7 days	--	--
"M8"	--		0.89 ppm (8.9%) @ 30 days	--	--

Notes:
(a) Major degradates (.10% of applied) are given in **bold type**.
(b) Data from the Photodegradation on Soil study are provided to show qualitative similarity to the Photodegradation in Water study. The half-lives from the former study are questionable, therefore the timing of maximum degradate concentration is also questionable.

Figure 2. Structures of PBO Degradates and Degradation Pathways.

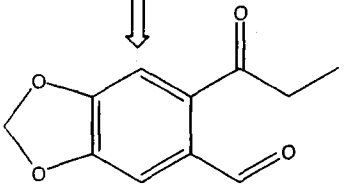


PBO Degradation: Aerobic Soil
Pathway 2



PBO prop-1-one

"M16"

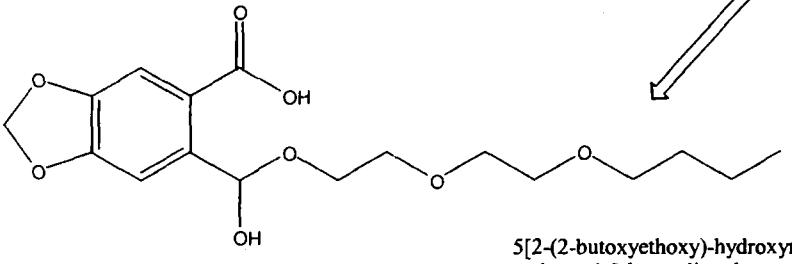


PBO prop-1-one benzaldehyde

"M11"

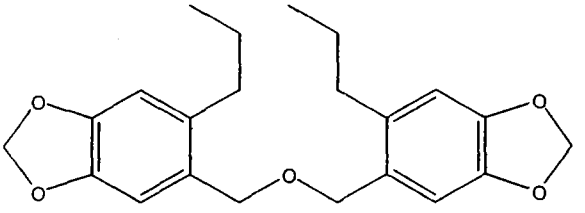
PBO

PBO-acid



5[2-(2-butoxyethoxy)-hydroxymethyl]-6-carboxy-1,3-benzodioxole

"M8"



PBO-alcohol
(Soil Photodeg. only)

PBO-alcohol dimer

13544

R113530

Chemical:	**Piperonyl butoxide**
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